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RESEARCH NOTES

Annan, Murvel E. Effects of dehydration on X-ray-induced damage to Drosophila females.

Virgin D. melanogaster (Oregon-R) females, 24 to 48 hours old, were placed either in a desiccating chamber or in a moist chamber. After 8 hours of this treatment, they were exposed to 5000, 2500, or 0 roentgens of X-rays. Immediately after exposure, each female was placed in an individual container with two males. Every day following treatment (for 10 days), two individual females from each of the six treatment groups (2 degrees of dehydration and 3 degrees of irradiation) were selected randomly to be sacrificed for ovarian examinations. The ovaries were examined for quantitative enumerations of stages 5 and 10 of oögenesis (after King, et al., GROWTH, 1956) for 10 days following treatment.

The data are too few to permit drawing general conclusions on the effects of X-rays on frequencies of stages of oögenesis. In none of the data were there any significant deviations due to the dehydration treatment. Herskowitz (DIS, 1956) found that dehydration increased X-ray-induced egg mortality. Although the deviations due to dehydration in our results were not statistically significant, the mean values were in the opposite direction; that is, dehydration tended to decrease egg mortality. This failure to duplicate Herskowitz's results may have been due to our use of a different degree of dehydration or different-aged females. Further work is under way to find possible effects of degree of dehydration on X-ray-induced damage to D. melanogaster females.

(This research was supported by U.S.P.H.S. grant RG-4626.)

Aslaksen, Erik. Pleiotropic pattern of the mutant red (red Malpighian tubules) in D. melanogaster.

The mutant red used in this experiment was received from I. Oster, and the investigations were carried out together with E. Hadorn in Zürich. We found that the red eye pigment and the content of isoxanthopterine are decreased in this mutant compared to what is found in the wild type. This is compensated for by an increase of other pterines. The red pigment in the Malpighian tubules is found to be an ommochrome, but is not the same as that found in the eyes. In experiments on transplantation of eye discs, the mutant red exhibits autonomous development with respect to the amount of red pigment.

Auerbach, C. The production of visible mutations by chloroethyl methane sulphonate (CB 1506).

In view of the personal factor involved in the scoring of visible mutations, it seems important to obtain independent information of the claim that some alkylating agents produce very high frequencies of visible mutations, many of which have not occured previously. In Muller-5 experiments by the Fahmys, CB 1506 produced an over-all ratio of 4 visible to 10 lethal recessives, and in later broods from sensitive spermatogonia this ratio increased to near unity. In the experiments to be presented here, 1- to 3-day-old OrK males were injected with a 10^{-2} M solution of CB 1506. They were then mated to a succession of virgin females at intervals of 3-4 days. In broods d (days 11-14) and e (15-18), half the

males were mated to 3 M-5 females each and half to 3 XX= females each; males which had been given one type of female in d were given the other type in e. The sons by XX= females were carefully examined by myself at 10x magnification, and all deviants were progeny tested. The F_2 vials of the Muller-5 tests were examined without etherization through a binocular. This was done by a laboratory assistant, who was told to look carefully for visibles. She was chosen for the job because she has had over 10 years' experience with flies, uses a higher magnification for scoring lethals than is customary in our laboratory, is unusually observant and as a rule detects more visibles in M-5 tests than I do. A summary of the results is tabulated. The figures in brackets refer to males which for some reason or other could not be progeny tested. The column "Exp. l" states the number of lethals which, on the basis of the parallel M-5 test, would be expected in a progeny of the size sampled.

	♀♀ M-5				♀♀ XX=			
Brood	n	l	Vis.	% Vis.	n	Exp. l	Vis.	% Vis.
d	386	21	2	0.5	864	47	6 (8)	.7 (1.6)
e	379	50	2	0.5	878	115	27 (3)	3.2 (3.4)

In the Muller-5 tests the ratio of visibles to lethals was about 1 : 17, that is, within the range found in X-ray experiments in which similar scoring methods were used, including experiments carried out by us. It is very much below the 4 : 10 ratio found by the Fahmys. In the XX= tests the percentage of visibles was higher, presumably for two reasons. (1) Even very slight abnormalities could be detected. (2) Many of the detected mutants had poor or very poor penetrance in progeny tests, and these would have excaped notice in M-5 tests. In brood e, only 10 of the tested mutants were fully penetrant, and if these alone are used the percentage of visibles drops to 1.1. My results, therefore, do not confirm the finding by Fahmy and Fahmy. This does not, of course, invalidate their observations; but it shows that definition of what constitutes a visible mutation, methods of scoring, and powers of observation differ considerably between laboratories and workers. Judged by my results in X-ray tests, my standards are roughly similar to those used by the earlier X-ray workers. That the Fahmys used higher standards is shown by the fact that in X-ray experiments by the Muller-5 method, in which the F_2 cultures were "scored under a low-power binocular without etherization rather early after emergence," they found the same ratio of 2 visibles to 10 lethals which Spencer and Stern obtained in experiments in which every culture was etherized and examined under the binocular. Incidentally, it will be of interest to see the detailed data on which the Fahmys base their conclusion that the ratio of 2 visibles : 10 lethals in their X-ray experiments differs significantly from the over-all ratio of 3 visibles : 10 lethals in experiments with phenyl alanine mustard. According to the table published in DIS-28, this latter ratio fluctuates between 1.8 : 10 and 3.3 : 10, when series containing less than 400 tested chromosomes are excluded. An additional source of discrepancy between the findings of the Fahmys and myself may be that I counted visibles which occurred in clusters as so many single mutations, because obviously in these broods the nonmutated germ cells may also have occurred in clusters. The Fahmys, on the other hand, counted each cluster as a single mutation and corrected the frequency of lethals "on the basis of the frequencies of

identical visibles in each brood." If this correction was done on the basis of the largest observed cluster it must have resulted in an underestimate of the lethal frequency. Moreover, lethals, many of which are subject to germinal selection, might be expected to form smaller clusters than visible viable mutations.

The nature of the visible mutations which occurred in the experiment with CB 1506 was not striking. There were alleles of fu, f and w, and in addition a number of unidentified mutations to rough eyes, smaller body size, and spread or drooping or incised wings. The one striking fact was that two mutations occurred repeatedly in the progeny of different males. One was a satsuma-like allele of w, which occurred twice. According to an analysis, kindly carried out by Dr. M. M. Green, the two mutants are indistinguishable phenotypically and in their interactions with "zeste" and with the enhancer of w^e; they are thus probably identical. The other case was that of a mutation to rotated abdomen (not yet located accurately), which occurred twice independently in the experiment described above, a third time in another experiment with CB 1506, carried out on a different strain of males, and a fourth time in a recent experiment with mustard gas. These recurrences point to the possibility that some mutational steps occur more readily than others under the influence of CB 1506 and, possibly, of other mutagens with chloroethyl groups.

Auerbach, C., and Sonbati, E. M. The brood pattern of mutations after treatment with chloroethyl methane sulphonate (CB 1506).

Fahmy and Fahmy found that injection of CB 1506 into adult males produces very low mutation frequencies in early broods and very high ones in late broods. Possible causes of this unusual brood pattern are slow action of the chemical, long mutational delay, dependence of the mutagenic action on cell division or on rate or type of metabolism. These possibilities have been explored in a number of preliminary experiments. (1) Treated spermatozoa were stored in males or inseminated females, and mutation rates were scored (a) in progeny obtained during the first days after treatment, (b) in progeny from stored spermatozoa, (c) in progeny from males which had been kept with females for the periods of storing used in group (b). Mutation rates in (b) were intermediate between those in (a) and (c). Slow mutagenic action and/or mutational delay are thus only contributory causes of the brood pattern. With periods of storing exceeding 12 days, mutation frequencies dropped again, presumably because of selection against males or spermatozoa which had absorbed more than the average amount of mutagen. Possibly the slight drop in mutation frequency which the Fahmys obtained in the fourth brood may have a related origin, for with their slow-breeding method (1 ♀ per ♂ every 3 days) some storing of treated germ cells would appear unavoidable. (2) Immature inseminated females were injected, and progeny was collected (a) during the first 4 days, (b) from females which previously had been kept on sugar-agar for 1 or 2 weeks. Mutation frequencies in the female germ cells were the same in all series (about 7%). Thus oögonia respond immediately to the treatment, and there is no noticeable delay in the occurrence of the mutations. (3) Immature virgin females were injected and kept on sugar-agar or maizemeal-molasses food for various periods of time before mating and production of progeny. As in the previous experiment, storing on sugar-agar had no influence on mutation frequency. Storing on food resulted in an increase in mutation frequency

which after 2 weeks reached statistical significance. Thus, active metabolism seems to favor the mutagenic action of CB 1506. When successive broods were produced from the treated females, mutation frequencies tended to drop, sometimes drastically. It has not yet been decided whether this was due to lower mutation frequencies in young oögonia or to selection against more heavily affected cells. (4) If active metabolism should, indeed, promote the action of CB 1506, one would expect high mutation rates from the germ cells in well-fed larvae. This expectation was borne out in a number of small-scale tests. The most effective treatment so far consisted in keeping 72-hour-old larvae for 24 hours on food containing 10^{-2} M of CB 1506. 223 X chromosomes from treated males carried 80 lethals, and 613 X chromosomes from females carried 117 lethals.

Barigozzi, C., Castiglioni, M. C., and Di Pasquale, A. Genetical analysis of a new tumorous stock, melanotic e 144 in D. melanogaster.

A new spontaneous stock carrying pseudotumors (courtesy of Dr. E. Goldschmidt, Jerusalem) has been submitted to the same investigation as several other stocks in this laboratory. It proved to have its main group of genes controlling the production of pseudotumors on the second pair of chromosomes, although the first and third pair interact in raising the incidence. The stock, if selected for and against tumor manifestation, shows a strong homeostasis. High-incidence lines give only a small number of offspring, so that selection after 6 generations becomes difficult.

Bertram, Cl., Hohne, G., and Kunkel, H. A. Induction of sex-linked recessive lethals in D. melanogaster by high-energy electrons and conventional X-rays.

The following data are the result of new comparative investigations with 200-kV X-rays and fast electrons of a 15-MeV betatron (Fa. Siemens-Reiniger). Adult males of D. melanogaster (Berlin normal inbred), 3-4 days old, were irradiated and 24 hours later mated to Muller-5 virgin females for three days. The results shown in the table demonstrate that the rates of mutation are nearly the same after irradiation with equal doses of X-rays or of fast electrons. The mean lethal mutation rate per 1000 rep is 2.47 ± 0.24% for X-rays and 2.52 ± 0.28 for fast electrons. Under the described circumstances the relative biological efficiency of the high-energy electrons is within the range of the statistical error.

	Dose in rep	No. chromosomes tested	No. of lethals	Per cent mutation
200 kV X-rays	4000	2934	293	10.0
	2000	1373	66	4.8
15 MeV electrons	4000	2289	219	9.6
	2000	1019	57	5.6

Binder, R. G. Observation on the mite Histiostoma laboratorium in Drosophila cultures.

A population study was made of the Drosophila food mite, H. laboratorium, from November 1954 to June 30, 1955. Population growth curves were recorded under conditions of light (sun or artificial), dark, heat (up to 105° F), cold (40°-45° F), dryness (74%-86%

normal food weight), and moistness (water added to 400% normal food weight), tested singly or in combinations in 150 cultures. Observations were made with (a) reused Drosophila cornmeal-agar medium previously infested with mites, and (b) fresh medium inoculated with bacteria cultured from previous mite-infested food plus a few roccal-sterilized mites. The results were as follows:

(1) The mite population curve was ⌒ -shaped under normal viability conditions. The early plateau may indicate the bacterial incubation period (2-6 days after start of culture). The humps may indicate relationships between mite-hatching potential and available-food potential. (2) Mite populations were generally found to thrive in the presence of healthy living bacteria, especially when autolysis occurred. Many bacterial species contributed to the mite culture. (3) Population was found highest with alternating periods of light and dark at room temperature. Cold (40°-45° F) and particularly warm (near 100° F) cultures were almost inviable, though eggs could last for months. (4) The major limiting factors were available food and living space (cultures were in test-tubes with 1 g or 0.5 g of food), with the accumulation of waste a probable secondary factor. (5) Rise in pH was generally associated with population rise. pH 3.8-9 was associated with heavy population and escaping hypopi--which, under pressure of numbers might even bore out of waxed-in cotton plugs.

It was found that H. laboratorium can live on peptone food products, including beef-extract; but Drosophila cultures are especially favorable because the fly serves as a transport host. A mechanism sensitive to the shadows of passing objects causes the mite hypopus (the 6th of 7 epimorphic stages) to spring up and attach to passing objects with its ventral suckers. This can be demonstrated by passing a small brush above a bottle cap infested with hypopi.

These observations were made while the author was a graduate student in the Department of Biology, Purdue University. (Present address: 189 Staples Street, Farmingdale, New York.)

Bonnier, G. Effects of selection pressure on irradiated populations of D. melanogaster.

From a common wild-type stock which was made co-isogenic and homozygous for chromosomes 1, 2, and 3, different populations were started. All populations are kept in an incubator room at 25° C. In each of the populations the larvae are bred in vials (see below). After eclosion the adults are transferred to population cages, and thereafter all flies are given an acute X-ray dose of 1500 r. In two of the populations, named P 25 and P 200, the females then oviposit on blackened food in Petri dishes. This oviposition takes place from 5 p.m. to 9 a.m. the next morning, and, after 28 more hours, freshly hatched larvae are collected and put into 90 x 25 mm vials, with ordinary cornmeal-agar food. The amount of food is not weighed, as we think that differences necessarily must cancel each other. Twenty-five larvae are put into each of the vials of population P 25; and 200 larvae into the vials of P 200. Between 5000 and 6000 larvae are collected and transferred to the vials in this way. In order to get that many larvae it is often necessary to make the same kind of collection for two or even three days in succession. When the adults emerge in the vials the same procedure is repeated for the next generation. In this way

every generation has a length of exactly 3 weeks. Two other populations, bb 25 and bb 200, are handled in the same was as P 25 and P 200, respectively, except for the way in which the oviposition takes place. In order to have a guarantee that no single female will produce too large a number of offspring, the egglaying is done in vials. Five females, together with males, are put into each vial, which contains a spoon with blackened food; no more than 25 larvae are collected from any one of these vials. From population P 200 a new population P 400, with 400 larvae per vial, has recently been started.

These experiments have now been going for about one year. Different kinds of tests have been made. We expect that the first group of results will be available in the middle of 1958.

Bonnier, G., Ramel, C., and Jonsson, Ulla B. Relative growth rates of larvae homozygous and heterozygous for alleles of the w locus.

Strains of five w alleles, namely, w^+, w^{co}, w^a, w^t, w, were made co-isogenic and homozygous for chromosomes 2 and 3 and for a part of chromosome 1 extending from a point between the loci of w and ec to a point to the right of the locus of f. Freshly hatched larvae were transferred to vials, 200 to each vial. In a first experiment, in which the growth rates of larvae of the pure strains were compared, each vial got 100 larvae from one of the strains and 100 from another strain. On the average 83 per cent of the larvae reached eclosion in a practically 1:1 proportion between the two competing types. However, at the time when only about 25 per cent of the larvae had reached eclosion, great differences were found between the two competing types. For one thing, it was found that w^{co} grew at a slower rate when competing with w^+ than when competing with w^a, and w^a grew at a slower rate when competing with w^+ or w^{co} than when competing with w^t or w. In a second experiment, comparisons along the same lines were made between the growth rates of homozygous and heterozygous wild-type females (which were identified by progeny tests with regard to their genotypic constitution). It was found that in competition between homozygotes w^+/w^+ and any of the heterozygotes, w^+/w^{co}, w^+/w^a, w^+/w^t, w^+/w, the homozygotes grew faster than the heterozygotes, New experiments are in progress.

Brown, Wm. P., and Bell, A. E. Analysis of developmental time involving three isogenic lines of D. melanogaster.

Three randomly chosen wild-type isogenic lines, which had been isolated from a closed population that exhibited a plateau for fecundity after 47 generations of selection (Brown and Bell, DIS-29), were carried for 25 generations by sib mating without artificial selection. Subsequently, a new isogenic line was derived from each of the three lines, thus giving six lines which were utilized in the analysis reported here. Crosses between the lines were made in all possible combinations, including pure lines and reciprocals, and a 24-hour egg deposit was collected from each. All progeny from each cross were observed and classified for sex, and the time of emergence was recorded. The time of emergence was broken down into 10 periods of 12 hours each (midnight to noon, and noon to midnight), making an emergence duration of 120 hours. A mean emergence time was

obtained for each sex and cross, and a constant of 240 hours representing the time between oviposition and the emergence of the first fly was added to the average emergence time to obtain the average developmental time. An analysis of variance revealed a highly significant difference in average developmental time between the sexes, with 288 hours for females and 294 hours for males. A highly significant difference was also found between crosses. When the crosses were summarized according to source of the parents, the following relationships were obtained.

Type of crosses according to source of parents	Average developmental time in hours
1. Among original lines	287
2. Between original and derived lines, excluding each original and its derived line	290
3. Between each original and its derived line only.	290
4. Pure original lines	293
5. Among derived lines	295
6. Pure derived lines	296

Since the original lines were carried for 25 generations without selection, mutations no doubt occurred within each which made them no longer isogenic. Assuming that earlier emergence is an indication of vigor or heterotic effects and that in general the degree of probable heterozygosity decreases as one goes down the table, the summary suggests that vigor in terms of developmental time is directly related to the degree of heterozygosity. A more detailed analysis of these data is under way.

Burdette, W. J. Interaction between tumor genes in Drosophila and genoid for CO_2 sensitivity.

Evidence that germinal mutants control cancer susceptibility is well established, but examples of tumors associated with viruses are increasing in number. As part of an investigation into the relations between the two, chromosomes bearing tumor genes were introduced into cytoplasm containing the genoid, Tr, for CO_2 sensitivity. The first table demonstrates a uniform distribution of the agent in individuals with and without tumors in the experimental group. The second shows a decrease in incidence of tumors in comparisonwith control cultures. The genoid apparently alters tumor incidence in the presence of susceptibility genes, although it neither incites tumors alone nor induces heritable changes at these loci.

CO_2 Sensitivity tu vg bw

	Males			Females			Total		
	Sensi	Total	%	Sensi	Total	%	Sensi	Total	%
With tu	895	899	100	801	804	100	1696	1703	100
Without tu	117	119	98	201	202	100	318	321	99
Total	1012	1018	99	1002	1006	99	2014	2024	99

Effect of Genoid on tu vg bw Tumor Incidence

	Males			Females			Total		
	tu	Total	%	tu	Total	%	tu	Total	%
CO_2 Genoid	1555	1753	89	1466	1803	81	3022	3556	85
Control	604	608	99	546	546	100	1150	1154	100

(Aided by a grant from the National Cancer Institute.)

Burla, H., and Zürcher, C. Color polymorphism in D. melanogaster.

In females of a wild strain from Uganda, obtained through the courtesy of Dr. Dobzhansky, the dark marginal bands of the abdominal tergites are broadened and the light anterior parts of the tergites are shadowed, a condition uncommon in the species, but similar to that in dark females of D. kikkavai. Densimetric determination of the darkness of selected parts of the tergites gave no overlapping between the two color types. The trait appears to show simple monohybrid inheritance with incomplete dominance, the heterozygotes showing intermediate darkening of the tergites. Dark homozygotes have reduced viability in comparison with light and intermediate phenotypes.

Castiglioni, M. C. Developmental genetics of pseudotumors in D. melanogaster.

A developmental study of pseudotumors in D. melanogaster has been carried on in close collaboration with the formal investigations of Barigozzi and Di Pasquale. (see DIS-28,-29,-30). It has been possible to show that the presence of a polygenic tu system is not sufficient to explain the developmental behavior of the character; thus it is necessary to postulate the existence of another polygenic system, modifying or conditioning the action exerted by the tu genes. More than 20 different genotypes have been studied, and it has been concluded that the presence of melanotic masses is the result of at least two independent mechanisms: (1) A more or less apparent disruption of the first pair of lymph glands during the third instar, with the consequence that a large number of cells-previously contained in the gland-swim freely in the hemolymph. One kind of these cells, especially, tends to congregate in clumps. (2) Melanization of the clumps, and finally, disappearance of any cellular structure. These two mechanisms combine in the following way:

Disrupted gland		Preserved gland	
a		b	
tumors	.....	no tumors	..... ability to melanize
c		d	
no tumors	.....	no tumors	..... inability to melanize

Condition a occurs in all tumorous individuals in every genotype. Condition b is typical in the tumorless individuals of low-incidence stocks. Condition c occurs in some stocks without tumors (e.g., +/+ +/+ Sb Me/H) and in those cases where tu genes have been replaced by their alleles through recombination. Condition d occurs in some wild stocks (Varese) as well as in heterozygotes of this type: Varese/tu stock. These observations have been made with three techniques: (a) examination of the whole gland, (b) examination of a series of microtome sections, (c) counts of the hemolymph cells smeared and stained with May-Grünwald Giemsa. The results obtained by these techniques have always been in good agreement. The polygenic system controlling the disruption or the preservation of the lymph gland during development works epistatically upon the tu system.

Crowell, Villa E. B., and Herskowitz, I. H. Heterosexual activity and longevity of the Drosophila male.

Although it has been frequently noted in the literature that unmated females live longer than mated ones, the effect of mating on the longevity of males has not been sufficiently studied. Loeb and Northrup (1917) found that at 30° C "isolated males lived a little longer than males when mixed with females," the noncelibate males living only 83.5% as long as celibate ones. To prevent isolation from being a possible factor, the effect of heterosexual activity of males on their longevity was studied as follows.

Oregon-R males less than one day old were collected and placed, one per vial, in vials containing standard yeasted food medium. In class I (110 flies), one virgin of a type easily distinguished from the wild type was also placed in each vial. In class II (114 flies), one Basc male fly was added to each vial. Every day for the first 11 days and every other day thereafter the flies were transferred to fresh vials. In class I the male was separated at each transfer, without etherizing, from the old female and put into a vial with a fresh virgin. In class II a simple transfer was made to a fresh vial with a fresh Basc male added if the old one was dead. The used vials of class I were kept for a few days to check on whether mating had taken place (by the presence of larvae). The experiment was carried out in an air-conditioned laboratory with temperature at about 24°-25° C.

The mean lengths of life for the classes of flies were: I (mated flies), 51.0 days; II (unmated flies), 56.8 days. The percentage of fertile matings decreased gradually for the first seven weeks, after which it declined rapidly. After 58 days only one fly produced offspring. The averages indicate that under these conditions the mated males lived about 90% as long as the unmated males. However, at the end of two months, 23.6% of the mated flies were alive as against 20.2% of the unmated flies. Thus, although the mated males died off earlier than the unmated ones during the first two months, toward the end of the experiment the mortality of the unmated males may have become greater than that of the mated ones. This has been indicated more strongly in two other comparable experiments in this laboratory.

(The work was supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT(11-1)-195.)

Di Pasquale, A.
Reappearance of pseudotumors in tumorless isogenic recombinants.

From tumorous stocks of D. melanogaster (tu A2 and tu B3, see DIS-28,-29,-30) isogenic recombinants for the second chromosome were obtained, without tumors. Note that the involved genes (tu) are located mainly on the second chromosome. The aim of making recombinants isogenic was to be able to keep them for further studies. After an interval of about 36 generations, pseudotumors began to reappear. From each stock 5 individuals were isolated, and the descendants again made isogenic, using a Cy L/Pm stock, which suppressed crossing over very well (0.5% between arms, no crossovers for the tu region, located at the right end, out of 832 individuals; the marked chromosome was cn c px). The new isogenic lines showed pseudotumors, whose frequency increased in the following generations. The reappearance of pseudotumors, as well as their increase in isogenic lines, are phenomena difficult to interpret. Excluding incomplete suppression of crossing over (because of the stock used), two possible explanations remain: (1) a cytoplasmic agent, acting like an infecting particle; (2) high frequency of mutation of the tu genes. At present, there are no reasons to prefer one of the two possibilities.

Dobzhansky, Th., Mallah, G. S., Tantawy, A. O., and Mourad, A. M.
Collection of Drosophila species in Egypt.

A survey collection of Drosophila fauna in different localities in Egypt was made between July 1956 and October 1957, mainly during the summer months. A few species, differing in their relative frequencies were captured. These were D. melanogaster, D. simulans, D. buzzati (repleta group), and D. busckii; the last species was very rare. Collections were made by means of fermenting banana traps from the following regions: University of Alexandria Farm (UF), Alexandria; Abou-Sir (AS), 40 km west of Alexandria; Fayuom (FY), 92 km southwest of Cairo; Beni-Swef (BS), 112 km south of Cairo; Kom-Ombo (KO), 640 km south of Cairo; Mehalla-El-Koubra (MK), 128 km east of Alexandria; Wadi-El-Natroon (WD), 90 km south of Alexandria; Noubaria (NB), 30 km southeast of Alexandria.

The frequency distribution of adults of D. melanogaster and D. simulans captured is shown in the table. Other species are not listed because of their rarity.

Locality	Date of Collection	D. melanogaster			D. simulans		
		Males	Females	Total	Males	Females	Total
UF	August 1956	206	120	326	26	30	56
	October 1956	160	109	269	36	20	56
	December 1956	28	22	50	26	19	45
AS	August 1956	1	3	4	112	95	207
FY	July 1956	90	75	155	3	8	11
BS	December 1956	1	9	10	246	277	523
KO	August 1956	--	3	3	--	--	--
MK	July 1957	120	126	246	18	50	68
WD	September 1957	49	94	143	5	25	30
NB	August 1956	248	500	748	1	20	21

Most of the collecting was done during July, August, and September from grapes, figs, and palm dates. The results clearly indicate that D. melanogaster dominates D. simulans in some regions and D. simulans dominates D. melanogaster in other regions. For instance, the NB population consists mostly of melanogaster whereas the BS consists mostly of simulans. The UF results demonstrate that collections are more successful during the summer than in the winter.

The Department will carry out such collections for all the Drosophilidae fauna in Egypt in the very near future.

Ensign, S., and Miller, D. D. Sexual isolation in the affinis subgroup.

An effort was made to study sexual isolation between various species of the affinis subgroup by placing newly emerged flies together and allowing them to cohabit for 10 days. At the end of this time, the females were dissected and the ventral seminal receptacles examined for sperm. The following are old (ca. 1940), unpublished data of D. D. Miller, who used recessive mutant strains of the different species D. algonquin (droop wings), D. affinis (rugose), D. athabasca (vermilion and cinnabar), D. azteca (cinnabar), and D. narragansett (bubble):

Females	Males				
	aff.	alg.	ath.	azt.	narr.
aff.	50/51 (98%)	0/56	*33/52 (39%)	0/57	0/50
alg.	0/52	45/51 (88%)	*3/53 (6%)	0/52	5/56 (9%)
ath.	0/102	0/104	45/53 (85%)	*9/53 (17%)	0/56
azt.	0/52	0/61	*45/59 (76%)	49/53 (93%)	0/54
narr.	0/55	0/53	0/57	0/52	47/51 (92%)

Recently Ensign made the following observations with D. tolteca (Nicaragua) in conspecific and interspecific crosses involving D. affinis (Nebraska), D. algonquin (Nebraska), D. athabasca (Wyoming), and D. azteca (California):

Females	Males				
	aff.	alg.	ath.	azt.	tolt.
aff.	39/52 (75%)				16/55 (29%)
alg.		54/67 (81%)			0/71
ath.			49/55 (89%)		+4/57 (7%)
azt.				48/51 (94%)	*23/60 (38%)
tolt.	12/52 (23%)	4/55 (7%)	31/51 (61%)	24/55 (44%)	53/56 (95%)

*Adult hybrids, all kinds of which have already been reported elsewhere (Sturtevant and Dobzhansky, 1936; Miller, 1950; and Patterson, 1954).

+Very few larvae obtained.

Fahmy, O. G., and Fahmy, Myrtle J. Selective cell-stage response to the mutagenicity of S-chloroethyl cysteine in D. melanogaster.

Our analysis of the mutagenic cell-stage response during spermatogenesis to the alkyl-methanesulphonates (DIS-30; Fahmy & Fahmy, 1957b) has revealed a gross difference in the brood-mutation pattern of ethyl methanesulphonate as compared to its chloro-derivative (2-chloroethyl methanesulphonate). The ethyl ester was most active on the late germ cells (spermatozoa and spermatids) and practically ineffective on the early germ mother cells, whereas the chloroethyl ester gave maximal mutagenic effect on spermatogonia. Biochemical evidence was available that the chloroethyl ester could produce in vivo a metabolite S-chloroethyl cysteine, which is a monofunctional amino acid mustard. The mutagenic effect of this mustard was therefore investigated to determine its possible role in relation to differential cell-stage response.

S-chloroethyl cysteine was administered by injection into adult males of the same age and average weight as those used in the experiments with the sulphonates, and their progeny was fractionated according to our standard technique, that is, in 3-day broods. The yield of sex-linked mutations was determined by the Muller-5 method and that of autosomal mutations by the Cy/BlL technique. The results indicate an extraordinary differential mutagenic effect on the early germ cells, particularly the spermatogonia. An injected concentration of 0.27×10^{-2} molar, induces a recessive lethal rate of the order of 0.5% in the sex chromosome and 1.0% in the second chromosome of sperm and late spermatids, whereas the corresponding rates in spermatogonia rise to an average of roughly 15% and 40% respectively. With the cysteine mustard the high mutation rate in spermatogonia (predominantly recovered starting from the 5th brood, as indicated by visible clusters) cannot possibly be due to longer time of treatment, since the compound is almost completely inactivated by hydrolysis within 1 hour.

The selective action of S-chloroethyl cysteine on spermatogonia adds further support to our interpretation of the pattern of mutagenic cell-stage response under the effect of 2-chloroethyl methanesulphonate. It was suggested that the high mutation rate induced in spermatogonia by the latter compound is not a function of the sulphonate itself, but of a secondary metabolite chemically related to the cysteine mustard.

Farnsworth, M. W. Mitochondria in normal and nullo-X embryos of Drosophila.

Boell and Poulson (Anat. Rec. 75: 65, 1939) reported the results of studies on oxygen uptake in wild-type and nullo-X eggs of D. melanogaster. They found that YY eggs respired initially at the same rate as controls. Within several hours after egg deposition, however, the oxygen uptake of the genetically deficient embryos fell to a level one-fifth that of wild type. The time of respiratory breakdown was found to coincide with the onset of the developmental anomalies characteristic of nullo-X individuals. In view of the association of some respiratory emzymes with mitochondria, the question arose whether or not mitochondria are present in nullo-X individuals. Since the distribution of mitochondria in early cleavage and blastoderm stages of normal embryos has not been described, the present study was undertaken to determine the localization of these components in both normal and X-deficient embryos and to ascertain, if possible, whether or not these particles are active.

The stock of D. melanogaster used in the study was In(1)dl-49, ty-1 bbl/ y v f car. The attached-X females of this stock carry a free Y chromosome. They were crossed to Canton-S wild-type males and inbred for six generations before egg collections were initiated. One-fourth of the eggs produced by these females were expected to be nullo-X (YY). This class of progeny could be readily recognized in living, dechorionated eggs. The subsequent developmental history of these individuals agreed with that described by Poulson (J. Exp. Zool. 83: 271, 1940).

For the cytological demonstration of mitochondria in both normal and X-deficient eggs, Flemming's fixative without acetic acid was employed. Control embryos of both genotypes were preserved in Flemming's with acetic acid and in Kahle's fluid. In these groups, mitochondria could not be demonstrated. Approximately 400 embryos from fertilization to blastoderm stages were used. For in vivo work, Janus green B in Drosophila saline was employed. In an attempt to ascertain the presence of dehydrogenases, specifically succinic dehydrogenase, as an indication of mitochondrial activity, histochemical studies utilizing neotetrazolium chloride were carried out after the methods of Shelton and Schneider (Anat. Rec. 112: 61, 1952).

In normal embryos, mitochondria were found to be localized primarily in the protoplasmic islands surrounding the nuclei. The morphology of these particles was usually filamentous. At the anterior dorsal end of the egg near the region of the micropyle, a concentration of mitochondria was found outside the protoplasmic islands. This diffuse granular mass extended posteriorly about one-fourth the length of the embryo. It was most evident in early and middle cleavage stages and could no longer be observed at the time of nuclear migration. During blastoderm formation in the normal embryo, the mitochondria of the protoplasmic islands remain in close association with their respective nuclei. These particles can be identified around the periphery of the nuclear membrane. In later blastoderm stages, cell membranes form between nuclei and each cell so formed appears to contain its own complement of mitochondria.

In the nullo-X individual, the distribution of mitochondria follows the pattern of the normal individual. Mitochondria remain in association with their respective protoplasmic islands regardless of the abnormal distribution of those islands. With the onset of cell membrane formation, mitochondria were included in the cells. A comparative count of the number of mitochondria in normal and nullo-X cells could not be carried out because of the small size of the particles and the crowded condition of the blastoderm cells of both genotypes. On the basis of general appearance, however, the number of mitochondria in cells of the X-deficient embryos did not seem to differ markedly from that of the normal.

Janus green preparations of squashed blastoderms showed stained granules present in the cytoplasm of cells of both genotypes. The number of such granules was not significantly different in the two genotypes.

Reduction of neotetrazolium chloride occurred only in those eggs whose vitelline membranes had been punctured. No color reaction was obtained with material fixed in Kahle's fluid or incubated without the tetrazolium salt. The addition of succinate as substrate had no observable effect on the speed of the reaction or the depth of color obtained. Apparently, sufficient substrate is already present in the yolk of the early embryo. Under these

circumstances, it cannot be stated which substance is being utilized or what enzyme or enzymes are responsible for the reaction. In squash preparations, formazan crystals were found primarily associated with the nucleated protoplasmic islands, the site of the mitochondria.

The results of this study demonstrate the presence of mitochondria in cells of X-deficient individuals. The drop in oxygen consumption which coincides with the onset of developmental failure cannot be explained, therefore, on the basis of the absence or degeneration of these particles.

Frydenberg, Ove. Equilibrium of a lethal mutant.

A dominant mutant at the Sb locus was isolated approximately three years ago from a natural population of D. melanogaster in Wisconsin. Though the mutant is lethal when homozygous it has been observed to persist in stocks. Investigations have shown that the mutant is linked to an inversion in III-R, the inversion being of much the same extent as the Payne inversion with which it is possibly identical. Two sets each of 10 populations of the bottle type were set up in such a way that the generations were kept separate. In one of the sets the emerging flies were allowed to start egg-laying as they emerged. In the other set the flies were collected as they emerged and stored until all flies had emerged; the whole generation was then allowed to start egg-laying simultaneously. Selection in favor of rapid development was operating in both sets, but it was obviously stronger in the first set then in the second. In the populations of the first set the mutant reached equilibrium at gene frequencies between 20% and 30%, regardless of whether the initial frequency had been 2.5% or 50.0%. The populations in the second set established equilibria around 10%, again regardless of initial frequency. In three ordinary population cages in which the generations were not kept separate the mutant has dropped in frequency to less than 1% during nine months of observation, which probably means that it is being eliminated from the populations.

The equilibria in the bottle populations seem to be maintained by superiority of the heterozygotes, which have a slightly faster larval and pupal development than the wild type and whose males exhibit a greater readiness to mate during the first 4 to 6 days of adult life than the wild-type males do. However, in the big cages where flies of all ages are present at any one time the young heterozygous males can not compete successfully with the older wild-type males.

After several generations of distinct equilibrium the mutant was suddenly completely eliminated from three of the bottle populations. This fact remains unexplained, but it seems likely that a recombination which separated the mutant from the inversion was responsible.

Glass, B., and Ritterhoff, Rebecca K. Radiation-induced sterility after irradiation of third-instar larvae.

The results of treatment of male third-instar larvae with doses of 1000 r and 2000 r, previously reported by A. F. E. Khishin (DIS-29: 128, and Z. indukt. Abst.-Vererbl. 87: 97-112), have been fully confirmed in our own experiments. In addition, female larvae were treated, like the males at an age of 80 ± 1 hours after hatching, at 25° C. When given 2000 r, the males

were completely sterile until 4 days after eclosion, and at 7 days post-eclosion only one-third to one-half of them had recovered fertility. Fertility continued to increase to a maximum of about 70% of individuals. Among the correspondingly X-rayed females, on the other hand, fertility was significantly reduced only on the first and second days after eclosion. After a dose of 1000 r in the third-instar larval period, the females recovered fertility about as quickly and completely as after 2000 r; but the males showed a more rapid and more general recovery after the lower dose. Some males (ca. 3%) were fertile on the second or third day after eclosion; on the fourth day the percentage reached nearly one-half; by the seventh day 80% of individuals were fertile. These results indicate either that oögonia and spermatogonia, and young primary oöcytes and spermatocytes, differ considerably in sensitivity to X-rays at comparable stages, or, as Khishin has suggested, that much of the infertility induced in males irradiated as larvae is not attributable to killing of germ cells or induction of dominant lethals, but is due to induction of physiological changes that render the spermatozoa ineffective.

Glassman, E. Studies on maroon-like eye color mutant.

Previous work (Science 124: 725, 1956; Rec. Genetics Soc. 26: 372, 1957) has shown that both maroon-like (ma-l) and rosy (ry) eye color lack the enzyme xanthine dehydrogenase, and as a result accumulate the substrates of this enzyme (hypoxanthine and 2-amino-4-hydroxypteridine) and do not form the products (uric acid and isoxanthopterin) formed from these compounds. Ma-l is a sex-linked recessive recently relocated to the left of Bx (see below), and ry is on the third chromosome at 51±.

Hadorn and Schlink (Nature 177: 940, 1956) have shown that ry is non-autonomous when appropriate transplantations of tissues and anlage are carried out. Since ma-l resembles ry, autonomy of ma-l was tested by crossing aged y ec ma-l; st females to X^{c2}, cv v f; st males, according to the method of Hannah. One gynandromorph was produced which exhibited y, ec, and a smaller male-like wing and sex combs on the left side; the right side was wild-type (or female) with respect to these characters (external genitalia were male-like). However, the color of the eyes on both sides was scarlet (ma-l; st is yellow-orange), indicating that ma-l, like ry, is nonautonomous.

In most crosses ma-l acts as a typical sex-linked recessive. However, ma-l/ma-l^+ females do not produce phenotypically ma-l progeny until the bottle is 16 to 20 days old. For example, if m ma-l/TM6; st females are crossed to m ma-l; st males, the non-TM6 males and females are phenotypically st, although progeny tests indicate that they are genetically ma-l (the difference between ma-l and ma-l^+ is more pronounced if st/st is present). A similar result is obtained if y f:=; st females are crossed to m ma-l; st males. This maternal effect from females containing ma-l^+ continues through the first 6 to 10 days of hatching; after this time the adults that hatch are typical orange-eye ma-l; st progeny. This reversal indicates that the maternal effect is also affected by a food factor, which either is taken up by older larvae, or is an inhibitor accumulating as a result of larval action. That the female parents themselves do not exhaust some chemical in their bodies is evidenced by the fact that seven successive transfers of y f:=; st females (crossed to m ma-l; st males) to new food bottles after 5 to 7 days

produced maternally affected male offspring (i.e., st eye) in the first 5 to 6 days of hatching, and typical orange-eye ma-l; st males thereafter in each bottle. Cross-feeding from ma-l^+ to ma-l larvae has been eliminated by raising ma-l^+ and ma-l flies in the same bottle; no effect of ma-l^+ on ma-l was observed. It is interesting that ry^+ does not have a maternal effect in the ry/ry^+ heterozygote. Studies on the biochemical changes associated with the maternal effect and the factor in the food may increase our understanding of the biogenesis of the red eye pigment.

Graf, G. E., and Hadorn, E. Chromatographic studies on Drosophila testes.

Paper chromatographic studies have been carried out on the testes of several species of Drosophila. Preliminary results indicate that the red pigments in the testes of D. subobscura and D. pseudoobscura are identical with the red pigments (drosopterines) found in the eyes of D. melanogaster. The pattern of other fluorescent substances is qualitatively similar but there are marked quantitative differences. The testes of both D. pseudoobscura and D. melanogaster are very rich in isoxanthopterine, whereas D. subobscura has relatively little of this compound. The testes of D. hydei resemble those of D. melanogaster, both qualitatively and in the relative amounts of the various fluorescent compounds.

Hexter, W. M. A mosaic resulting from double fertilization of a triploid female.

Single triploid females which contained attached-X chromosomes homozygous for g^{53d} sd and a free X which had the genes y^{31d} sc^8 w^a lz^s B (hereafter referred to as FM1) were fertilized by wy^2 g^3 males. From one such mating a female resulted whose left eye was g^{53d} (orange) and the left wing scalloped. The right eye was typical of a Bar heterozygote. This female, which was virgin, was mated by sn^3 males to test the possibility that her ovaries were genetically mosaic. She yielded the following progeny: females, 346 heterozygous Bar and 417 wild type; males, 421 wy^2 g^3, 298 FM1, and 2 sn^3. The sn^3 males proved to be sterile and presumably were nondisjunctional males. The heterozygous Bar females when mated yielded FM1 males, and the wild-type females when mated yielded wy^2 g^3 males. These results indicate that this mosaic female had nonmosaic ovaries which were genetically FM1/wy^2 g^3.

This mosaic female can best be explained by assuming that a double fertilization occurred. One female nucleus contained the FM1 chromosome and was fertilized by the wy^2 g^3 X chromosome of the male. This fertilization accounts for the right eye of the mosaic female and for her ovaries. The left eye and left wing most certainly resulted from a female nucleus containing the g^{53d} sd attached-X chromosomes fertilized by the Y chromosome. Another explanation, considered most unlikely, is that the egg nucleus contained both the attached-X and the FM1 chromosome (owing to nondisjunction) and was fertilized by the wy^2 g^3 chromosome. Then one would have to make the improbable assumption that as a result of the first cleavage one daughter cell lost the attached-X chromosomes and the other daughter cell lost both the FM1 and wy^2 g^3 chromosomes.

Hexter, W. M. Gynandromorphs probably resulting from double fertilization of attached-X females.

Single attached-X females, one X which was g^{53d} sd, the other wy^2 g^3, were mated by males which were y^{31d} sc^8 w^a lz^s B (hereafter referred to as FM1). From this mating two independent gynanders resulted. One was an almost perfect bilaterally symmetrical fly whose left side was yellow; the left eye was B lz^s w^a; and the front left leg had a sex-comb but no tarsal claw; the genitalia of the left side were male-like. The right side was non-yellow; no sex-comb was present on the front right leg although a tarsal claw was present; the eye was g^{53d} and the wing was scalloped; the genitalia of the right side were female-like. This gynander probably resulted from a double fertilization. One egg nucleus contained the attached-X chromosomes homozygous for g^{53d} sd (resulting from a crossover of the attached-X) and was fertilized by a Y-bearing sperm, and the other egg nucleus contained the maternal Y chromosome and was fertilized by the FM1 sperm. Another explaination, considered improbable, would be the assumption that the gynander originated as a triplo-X and that as a result of the first cleavage one daughter cell lost the attached-X chromosomes and the other daughter cell simultaneously lost the FM1 chromosome.

From the same original cross another gynander was found whose genitalia were male although rotated 180 degrees. The left eye was garnet and the left front leg had no sex-comb but had a tarsal claw. The right front leg had a sex-comb and no tarsal claw. The right eye was B lz^s w^a with two small patches of apparently garnet facets. This gynander presumably arose as a double fertilization in the manner already described. Simultaneous elimination at an early cleavage of two different chromosomes, although unlikely, is not excluded.

Hiraizumi, Y., and Crow, J. F. The amount of dominance of "recessive" lethals from natural populations of D. melanogaster.

Newly arisen lethal mutants have been shown by Stern et al. (Genetics 37: 413) and by Muller and Campbell (unpublished) to cause about 4%-5% reduction in viability of heterozygotes. However, those mutants with the greatest heterozygous effect would be most rapidly eliminated from a population, so that the average effect of those lethals found in a natural population would be less. It can be shown (Muller, Morton, and Crow, PNAS 42: 855) that the mutants remaining in a population would have an average dominance equal to the harmonic mean of the original values, and in Muller and Campbell's data this was estimated to be roughly 2%.

Tests were made of 53 second-chromosome lethals, 64 semilethals, and 60 control chromosomes isolated from natural populations near Madison, for heterozygous effects on viability in crosses of cn bw females x cn/+ males. Comparison of the ratio of wild-type and cn offspring for the three classes gave a measure of the heterozygous effects. There was no significant difference between lethals and semilethals, the average selective disadvantage as compared with lethal-free chromosomes being .030. Since some chromosomes may carry more than one deleterious gene, a correction was made assuming a Poisson distribution; this led to an estimate of .026 for the selective disadvantage in the heterozygote of a recessive lethal or semilethal.

Horikawa, M. Growth, differentiation, and tryptophan metabolism in eye discs of D. melanogaster in tissue culture.

Eye-antennal discs and cephalic complexes obtained from mature third-instar larvae (95 hours after hatching at 25° C) were cultured in vitro in a synthetic medium (see DIS-30: 161), to investigate the effect of the metamorphic hormone upon growth, differentiation, and trytophan metabolism in the eye discs.

When the eye discs of Oregon and bw were cultured in the synthetic medium containing 5 mg/ml L-tryptophan, together with the cephalic complex of the same body, brown pigment was deposited in the eye discs after culturing for about 72 hours. On the other hand, when the eye discs were cultured in the same medium together with the cephalic complexes of ten bodies, brown pigment was deposited in the eye discs after culturing for only about 24 hours. Furthermore, when eye discs were cultured together with ten cephalic complexes in medium not containing trypotphan for about 48 hours, and then transferred to medium containing tryptophan, the eye discs showed more pronounced growth, differentiation, and pigmentation than in any of the other cases mentioned above, after culturing for only about 16 hours.

In hanging-drop cultures, ten cephalic complexes gave the optimum concentration of the metamorphic hormone in the culture medium; more or less than ten cephalic complexes proved to have a less favorable effect on growth, differentiation, and pigmentation of the eye discs.

Various combinations of the eye discs of one strain and the cephalic complexes of another strain, or the metamorphic hormone extracted from pupae of the silk worm according to the method of Butenandt, were cultured in the same medium. According to the results, the cephalic complexes of some strains seem to be classifiable into three groups on the basis of qualitative and quantitative difference of the metamorphic hormone. The first group possesses normal hormone activity affecting growth, differentiation and tryptophan metabolism in the eye discs. Oregon, bw, v, and cn belong to this group. The second group possesses normal hormone activity as regards growth and differentiation, but not tryptophan metabolism. The mutants w, v, bw, and cn, bw belong to this group. The third group possesses normal hormone activity on tryptophan metabolism, but not on growth and differentiation. B, bar-3, and Dp/In (3L)P, In (3R)C, Sb e l(3)e belong in this group. The last group is divided into two subgroups. The eye discs of B and bar-3 showed more pronounced growth and differentiation with the addition of ten cephalic complexes of Oregon and the metamorphic hormone extracted from the silk worm. The eye discs of Dp/In(3L)P, In(3R)C, Sb e l(3)e showed no better growth and differentiation when these substances were added.

Jacobs, M. E. Influence of desiccation on dopa oxidizing activity and amino acid levels in D. melanogaster.

When late larvae, just before pupation, were exposed to low humidities for four hours, dopa oxidizing activity was accelerated, and the levels of glutamic and aspartic acids were increased and that of alanine decreased as compared with siblings kept in moist containers.

Khishin, Aziz F. Studies on heat tolerance in Egyptian populations of D. melanogaster and other Drosophila species.

Wild stocks of D. melanogaster as well as other species found in Egypt are constantly being collected. The present study is being carried out with populations collected mainly during the summer months. Summer temperatures in Egypt vary according to localities, and range from about 30° C. in Alexandria to about 40° C. or more in Upper Egypt (daytime temperatures). Some collections have been made from localities which are more or less isolated; therefore the degree of inbreeding is expected to be higher in some strains than in others. The object of the study is to find out how high temperatures affect the flies, and whether or not there are differences in heat tolerance among strains from different localities. Experiments are being carried out in controlled incubators. Flies from an Oregon-K stock are used as controls.

Preliminary results show that at 40° C. flies are not killed after exposure for 35 minutes. At this temperature flies become almost inactive and look as if trying to recover from etherization. When taken out into room temperature (25° C.) they eventually recover completely. Males were examined for sterility, and most of them were fertile. In another experiment flies stood the treatment for 130 minutes. Flies can be kept at 35° C. for about 3 days and sometimes more. They deposit no eggs, however, and usually die after that period. Larvae of all instars can stand 35° C. for 24 hours. Late-third-instar larvae usually pupate, but no adults emerge. Eggs, if exposed for more than 9 hours, do not hatch. Flies seem to tolerate well a temperature of 33° C. They are active and more or less normal. They deposit eggs; eggs hatch in almost the normal percentage; and larvae grow and pupate; however, no adults have been obtained. It is observed that adults attempt to emerge but only succeed in dragging the head and thorax out of the pupal case. They are found dead with their posterior halve inside the puparium. The experiments are still under way.

Kikkawa, H. Genetical analyses of resistance to parathion in D. melanogaster.

Among various strains of D. melanogaster, Hikone-R and WMB (selected by Hiroyoshi for DDT resistance) showed the highest resistance to parathion. The LD50 for larvae of these strains was about 2 ppm, whereas that for other nonresistant strains was about 0.08 ppm. Genetical analyses showed that the major gene for parathion resistance was at a locus near 64.5 (to the left of sca and vg) on the second chromosome.

Various mutant strains, which were originally nonresistant but obtained the chromosome segment responsible for parathion resistance in Hikone-R by means of double crossing over, showed high resistance. Cross resistance to various insecticides such as DDT and BHC was also found in these resistant strains. Presumably, the major gene located on the second chromosome may have a common mechanism for resistance to various insecticides, that is, it may be included in the category "vigor tolerance" of Hoskins (1956).

Kim, K. W., and Paik, Y. K. Keys to species of Family Drosophilidae occurring in South Korea.

The following six keys will undoubtedly be modified as the collections progress.

(1) Key to the species of Genus Amiota

1. Mesonotum uniformly dark brown, without markings; legs not banded; 5X index about 1.5; middle orbital bristle about 2/3 size of first..... .....alboguttata Wahlberg.
Mesonotum with dark markings; legs distinctly banded; 5X index about 0.8; middle orbital bristles about 1/3 size of first; arista with three or four branches above near base, none below.....variegata Fall.

(2) Key to the species of Genus Leucophenga

1. Arista with dorsal rays only.....argentosa Okada.
Arista with both dorsal and ventral rays.....2
2. Wings clear.....3
Wings with cloudy markings.....5
3. Palpi very large and black (but in male, palpi small)..... .....magnipalpis Duda.
Palpi moderate in size.....4
4. Third to fifth abdominal tergites with caudal bands..... .....concilia Okada.
Second to sixth abdominal tergites with black spots, mesonotum silvery pollinose in male.....maculata Dufour.
5. Postvertical bristles much smaller than orbitals...ornatipennis de Meijere.
Postvertical bristles well developed, nearly as large as middle orbitals .....quinquemaculipennis Okada.

(3) Key to the species of Genus Mycodrosophila

1. Wings clear; mesonotum uniformly black; thoracic pleura with black patches; second to sixth tergite with black caudal bands, second to fourth tergite bands broadly interrupted at middle; male fore tibia and tarsi with long recurved upright hairs along anterior margin..... .....poecilogaster Loew.
Wings clear; mesonotum uniformly black; thoracic pleura without black patches; second to fifth tergite with black caudal bands, second to third tergite bands broadly interrupted at middle..... .....Mycodrosophila sp.

(4) Key to the species of Genus Microdrosophila

1. Orb_1 about 1/5 as far from orb_3 as from verticals; mesopleura with a distinct black longitudinal stripe; third costal section with heavy bristles on basal 4/5.....Microdrosophila sp 1.
Orb_1 about 1/2 as far from Orb_3 as from verticals; third costal section with heavy bristles on the entire length.....2
2. Abdomen black; mesopleura without black longitudinal stripe..... .....congestor Zetterstedt.
Abdomen black; mesopleura with black longitudinal stripe..... .....Microdrosophila sp 2.

(5) Key to the species of Genus Scaptomyza

1. Body yellowish gray; acrostichal hairs in 2 rows; one prominent humeral. .....graminum Fall.
Body grayish brown or black; acrostichal hairs in 4 rows; two humerals. 2

2. Body grayish brown; costal index about 3.5; third costal section with heavy bristles on basal 2/5.....disticha Duda.
Body grayish black; costal index about 3.0; third costal section with heavy bristles on basal 1/3.....polygonia Okada.

(6) Key to the species of Genus Drosophila

1. Preapical bristles are small or absent on the first and second tibiae.....2
Preapical bristles evident on all three tibiae.....8
2. Mesonotum with distinct longitudinal stripes.....3
Mesonotum without stripes.....6
3. Acrostichal hairs in 8 rows; mesonotum with five narrow longitudinal stripes, the median longitudinal stripe is bifid posteriorly.....busckii Coquillett.
Acrostichal hairs in 6 rows.....4
4. Crossveins clouded; mesonotum with 4 longitudinal stripes; oral margin and cheeks snowy white.....alboralis Momma & Takada.
Crossveins not clouded.....5
5. Mesonotum with 3 pairs of narrow longitudinal stripes..sexvittata Okada.
Mesonotum with 4 black longitudinal stripes, inner pair broader caudally and outer pair interrupted at suture.....quadrivittata Okada.
6. Arista with one branch below in addition to terminal fork; abdominal dark bands not interrupted at middle.....nokogiri Okada.
Arista with a few branches below; abdominal dark bands interrupted at middle.....7
7. Orb_2 minute, about 1/5 size of first.....D. sp. close to histrio.
Orb_2 about 1/3 size of first.....D. sp. of Hirtodrosophila.
8. Prescutellar bristles present.....9
Prescutellar bristles absent.....11
9. Body yellowish; wings somewhat brownish along costa....puncticeps Okada.
Body blackish; wings clear.....10
10. Acrostichal hairs in 8 rows; third costal section with heavy bristles on basal 4/5.....coracina Kikkwa & Peng.
Acrostichal hairs in 6 rows; third costal section with heavy bristles on basal 2/3.....rufifrons Loew.
11. Abdominal dark bands never broken in mid-dorsal line.....12
Abdominal dark bands usually narrowed or broken in mid-dorsal line...20
12. Body blackish.....13
Body yellowish.....15
13. Acrostichal hairs in 6 rows.....helvetica Burla.
Acrostichal hairs in 8 rows.....14
14. Orb_2 about 1/3 size of first.....bifasciata Pomini.
Orb_2 about 1/2 size of first; abdominal tergites entirely black.....D. sp. close to bifasciata.
15. Acrostichal hairs in 6 rows.....16
Acrostichal hairs in 8 rows.....17
16. Orb_2 minute, about 1/5 size of other two; third costal section with heavy bristles on basal 2/5.....magnipectinata Okada.
Orb_2 about 1/3 size of first; third costal section with heavy bristles on 1/2.....auraria Peng.

17. Pulpus with a few prominent setae.....melanogaster Meigen.
Pulpus with only one prominent seta.....18
18. Costal index about 4.0; male wings apically with black spots.....
.....suzukii Matsumura.
Costal index about 2.0; male wings apically without black spots.....19
19. An indistinct very small blackish spot present in the groove located on the posterior base of fore coxa; posterior paramere with basal branch very long.....lutea Kikkwa & Peng.
No such blackish spot present; posterior paramere with basal branch very short; body is of small size.....takahashii Sturtevant.
20. A pair of presutural bristles present.....testacea van Roser.
No presutural bristles.....21
21. Yellowish or yellowish brown species.....22
Blackish or dark brown species.....31
22. Abdominal tergites with varieties of black spots.....23
Abdominal tergites without spots, usually with bands.....24
23. Crossveins and wing tip clouded.....nigromaculata Kikkwa & Peng.
Wing tip not cloudy, posterior crossveins slightly clouded.....
.....transversa Fall.
24. A row of short stout bristles on lower apical part of each fore femur.
.....25
Fore femur without such a row of bristles.....26
25. Posterior crossveins and tip of longitudinal veins cloudy; one prominent bristle at apex of first costal section.....immigrans Sturtevant.
Tip of longitudinal veins not clouded; two prominent bristles at apex of first costal section.....D. sp. of immigrans group.
26. Second oral over 3/4 size of vibrissa.....27
Second oral 1/3 or less size of vibrissa.....30
27. Crossveins deeply clouded; third costal section with heavy bristles on basal 1/3.....D. sp. close to kuntzei.
Crossveins clear or slightly clouded.....28
28. Acrostichal hairs in 8 rows; abdominal bands become narrow and indistinct as they approach the lateral margin.....histrio Meigen.
Acrostichal hairs in 6 rows.....29
29. Third costal section with heavy bristles on basal 1/4; abdominal bands of two basal segments are darker and broader than those of other segments.....bizonata Kikkawa & Peng.
Third costal section with heavy bristles on basal 2/5; abdominal black bands broaden laterally.....D. sp. of quinaria section.
30. Third costal section with heavy bristles on basal 1/3; acrostichal hairs in 6 rows; arista with about 7 very short branches including minute fork.....makinoi Okada.
Third costal section with heavy bristles on basal 1/2; acrostichal hairs in 8 rows.....D. sp.
31. Mesonotum with black spots; carian sulcate; costal index about 3.0.....
.....repleta Wollaston.
Mesonotum almost dark brown.....32
32. Abdominal dark band broadly interrupted at middle..D. sp. of Drosophila.
Abdominal tergites uniformly dark brown, band interruption is obscure.
.....33
33. Costal index about 2.5; palpus with only one prominent apical bristle.
.....virilis Sturtevant.

Costal index about 3.0 or more.....34

34. Posterior crossveins slightly clouded; genital arch black, anterior and posterior margin of lower portion symmetrically convex, clasper with invariably 9 teeth arranged in a straight row..cheda Tan, Hsu, and Sheng.

Crossveins clear.....35

35. 5X index about 1.5; genital arch black, anterior margin of lower portion slightly concave, clasper with about 12 black teeth arranged in a shallowly concave row.....lacertosa Okada.

5X index about 1.0; genital arch brownish black, anterior and posterior margin of lower portion symmetrically convex, clasper with about 10 black teeth arranged in deeply concave row.....sordidula Kikkawa & Peng.

King, R. C. Experiments on the alkaline earth requirements of Drosophila. Even the most highly purified agars contain large amounts of Ca and Mg. The cellulosic polymer "Methocel" (U. S. P. grade), produced by Dow Chemical, is free of these contaminants. Ten grams of 4000 centipoise viscosity type Methocel are added to 100 ml of boiling-hot distilled water. The resulting suspension is cooled rapidly to 2° C and poured into sterile containers, where it will form a thixotropic gel. Drosophila larvae can now be fed on nutrients pipetted onto this gel.

The yeast Candida monosa (strain Y-96 Northern Regional Research Laboratory, Peoria, Ill.) grows well in an aerated fluid medium of the following composition (weight of components in g): dextrose, 5; $(NH_4)_2SO_4$, 1; KH_2PO_4, 1; KCl, 0.4; $MgSO_4$. 7 H_2O, 0.06; $CaCl_2.2H_2O$, 0.06; Inositol, 0.025; $FeCl_3$, 0.0025; $MnSO_4.1H_2O$, 0.0025; pyridoxin HCl, 0.0005; thiamine HCl, 0.0005; biotin, 0.000025; distilled water, 100. It should be pointed out that reagent grade $MgSO_4$ contains 1 Ca atom per 800 Mg atoms, and $CaCl_2$ contains 1 Mg atom per 1000 Ca atoms. Candida monosa will grow if Mg or Ca is omitted from the medium. Drosophila will complete its life cycle when fed on such Ca-deficient yeast, but not when fed Mg-deficient yeast. Drosophila will complete its life cycle when fed Mg-deficient yeast on an agar gel, since it can extract the alkaline earth metals bound in the agar.

King, R. C. Oögenesis in female-sterile mutants. A study was made of Feulgen-stained whole mounts of ovaries of females homozygous or hemizygous for any one of 23 female-sterile mutations. The mutant ovaries may be grouped into various categories on the basis of their cytological appearance. A. "Normal": (1) oc, (2) lz^s, (3) na, (4) ap^{56f} (not sterile), (5) bf, (6) sv^{de}. B. Ovaries tumorous: (7) fes, (8) nw^2, (9) fu, (10) fu^{ff}, (11) fu^{57a}, (12) fu^{57f}. C. Synthesis of Feulgen-positive material in nurse-cell nuclei retarded: (13) sn^{36a}, (14) ras^4, (15) rn, (16) fs 2.1. D. Yolk synthesis retarded or abolished: (17) dm, (18) ty, (19) ap^4, (20) mi. E. Mature eggs stored, not laid: (21) sta. F. Certain mutants are characterized by exceptionally high frequencies of fusions between adjacent egg chambers in an ovariole (22) cg] or between germaria and adjacent chambers [(23) cmp.]. Fusions of adjacent chambers occur less frequently in ras^4, mi, and the fu alleles; rarely in dm, fs 2.1, rn, and oc.

King, R. C., and Burnett, R. G. Effect of etherization upon oögenesis.

Under our conditions a 15- to 20-second etherization is sufficient to anesthetize Drosophila so that they remain unconscious for several minutes. We were interested to see whether prolonged etherization of young females would disturb oögenesis. We used Oregon-R females which were less than 1 hour old, and etherized them for 45-60 seconds. More prolonged exposures were lethal. Approximately half the flies had unexpanded wings. Records were kept of daily egg production for days 5 through 10. The flies laid about 30 eggs daily. No significant difference was noted between the 45- and 60-second series or between flies exposed while their wings were expanded or unexpanded. However, etherization caused about half the flies with unexpanded wings to fail to expand their wings within 10 days. Feulgen-stained whole mounts of ovaries of flies killed 5 or 10 days after ether treatment were examined. The ovaries appeared normal. Pycnotic egg chambers were observed very rarely (1/90). It thus appears that etherizations normally used in Drosophila work produce no permanent damage to the female reproductive system.

Kuroda, Y. Comparisons of tyrosinase activity in tumorous and nontumorus strains of D. melanogaster.

Tyrosinase activity in the body fluid of third-instar larvae (95 hours after hatching at 25° C) of some tumorous strains of D. melanogaster was measured by the manometric technique, and compared with that in nontumorous strains. Sixty larvae were dissected in 3 ml distilled water, setting the body fluid free in the medium. This diluted body fluid was chilled for 20 hours at 4° C., incubated for one hour at 25° C, and centrifuged. The supernatant was used as the enzyme solution. Tyrosinase activity was measured by following in a Warburg respirometer its oxygen uptake of enzyme preparation in M/10 phosphate buffer (pH 6.8) at 25° C, using M/100 dopa as the substrate.

The results of comparisons between the tumorous strains st tu, v tu, and tu-h, and the corresponding nontumorous strains st, v, and Oregon (wild) are shown below.

O_2 uptake (µl/hr/larva)

st tu	st	v tu	v	tu-h	Oregon
18.3	16.0	9.3	8.5	14.9	10.6

Tyrosinase activity was higher in the body fluid of the tumorous strains than in that of the nontumorous strains.

Lewis, Bonny Morgan. Formaldehyde-induced crossing over in D. melanogaster males.

Larvae in the first or second instar were transferred onto either 0.20% or 0.25% formalin food in a total of five different experiments. Controls were also transferred, but onto fresh medium without formalin. The larvae completed development in the same culture bottles

without further transfer. When adult males of the constitution My Gl + +/+ + Sb bx^D emerged, they were mated individually to untreated virgin females of the constitution My + Sb +/+ Gl + bx^D, and crossovers in the middle region in sperms were studied and compared with spontaneous crossovers in eggs. This followed the crossover-selector system of Whittinghill (Science 11: 377, 1950). At the end of 3 days, the males were transferred and remated to fresh virgin females for a period of 6 days.

Fertile test crosses were completed on 150 treated males and 111 control males. Male crossing over was found only in the middle or Gl-Sb region. Since any one of the four markers is lethal in the homozygous state, viable offspring would occur by one of two means: a wild crossover sperm in combination with any egg, and noncrossover sperm in combination with certain crossover eggs. The origin of these viable offspring could be diagnosed correctly both by phenotype and by subsequent breeding tests.

Of the 150 treated males, 15 produced a total of 52 crossover offspring, representing an estimated frequency of .00306 crossover sperm. From control males, one crossover offspring was produced, representing an estimated frequency of .00003 crossover sperm. Comparison of the control and treated series shows that both the percentage of males with any crossover offspring at all and the estimated frequency of crossover sperm were significantly increased after the formalin-food treatment. From the brood data, a wide range of germ-cell sensitivity was suggested. The recovery of clusters of recombination offspring indicated that crossing over probably occurred in spermatogonial cells. However, the signle recombinations might have had either a spermatogonial or a spermatocytic origin.

Lewis, H. W., and Lewis, H. S. Thermostability of tyrosinase from Canton and sable adults.

Young adult flies of the Canton-S strain grown at 25° and 15° C and sable flies grown at 25° C all have the wild-type phenotype, whereas sable flies grown at 15° C express the sable phenotype. Tyrosinase activity in these four classes of flies differs markedly. Assigning a value of 100 to the tyrosinase activity of sable flies grown at 15° C, the value for sable flies grown at 25° C is 77, the value for Canton flies grown at the lower temperature is 71, and that for Canton flies grown at the higher temperature ia 17. Since the effect of temperature during development is greater on the wild-type tyrosinase than on the enzyme system under the control of the sable locus, it seems possible that the enzyme from wild-type flies is more thermolabile than the enzyme from sable flies. It also seems possible that flies grown at the lower temperature produce a more thermostable enzyme, independently of the genotype. To test these ideas, heat inactivation experiments were performed on tyrosinase extracted from young adults of the four classes of flies. These tests showed that the heat inactivation pattern is the same in all classes. That is, with regard to the property of thermostability, the enzymes of all four classes of flies are identical.

Lindsley, D. L., and Edington, C. W. A screening method for recovering sex-linked recessive lethals whose expression is influenced by the Y chromosome.

For every four sex-linked recessive lethals recovered by the Muller-5 technique there is one that is missed because its expression is suppressed by the Y chromosome and the X-irradiated/Y males survive. These XO lethal, XY viable changes are of considerable interest and the following series of crosses is designed

to facilitate their recovery. Three essentially new chromosomes are used in the crosses, and they will be described first.

1) Ins (1)sc^{8L}, d1-49, sc^{S1R}, y^{31d} B f v: The essential features of this chromosome are that it is multiply inverted, marked with B, and deficient for sc. It is similar to the Binscy stock in the Indiana list except that it is sc^-; it might well be symbolized Biny.

2) y l^{259} w m f: The essential features of this chromosome are that it has a normal sequence, is recessively marked, and lethal in combination with a normal Y but viable in combination with sc^8.Y since l^{259} is in the region of yellow.

3) Ins(1)sc^{4L}, S sc^{8R}, y sc^4 B w^a sc^8: The essential features of this chromosome are that it is multiply inverted, marked with y, B, and w^a, and deficient for the bulk of the proximal heterochromatin. As a consequence of the heterochromatic deficiency, X-Y pairing is reduced and primary nondisjunction frequent; X-, Y-, O-, and XY- bearing sperms are formed roughly in the ratio 45:35:15:5. This chromosome is essentially y sc^4 M-5, which we symbolize scumy-5 = S-5. A similar chromosome has been used by Oster (DIS-30).

y/Y males are irradiated and crossed to Biny/y l^{259} w m f virgins (from Biny/y l^{259} w m f x y l^{259} w m f/sc^8.Y stock). The F_1 consists of: y/Biny (phenotypically y B) female; y/y l^{259} w m f (phenotypically y) female; Biny/Y, which is phenotypically sc^- and dies; and y l^{259} w m f/Y, which dies. Since the F_1 males die, the females will be virgin if the parents have been dumped. y/Biny females are selected and crossed individually to S-5/sc^8.Y males. The expected progeny from this cross are Biny/S-5 (phenotypically y sc B/B) female; Biny/S-5/sc^8.Y (phenotypically sc B/B) female; Biny/sc^8.Y, which is phenotypically sc^- and dies; and Biny/O, which is phenotypically sc^- and dies; also y/S-5 (phenotypically y B/+) female, y/S-5/sc^8.Y (phenotypically B/+) female; y/sc^8.Y (phenotypically +) male; and y/O (phenotypically y) male. The cultures are examined for the presence of + (XY) and y (XO) males. The presence of both classes indicates a nonlethal-bearing X chromosome; the absence of both classes indicates an orthodox sex-linked recessive lethal. Two additional classes of lethal cultures are possible, those in which the XO but not the XY males appear and those in which the XY but not the XO males appear. y/S-5 (or y/S-5/sc^8.Y) females from each lethal culture can be recrossed to S-5/sc^8.Y males to check the original classification. These females will be virgin in every case except the XY viable XO lethal cases where, if the XY males are fertile, the inviability of XO males can be confirmed by crossing + males to XX/O females. Finally, lethals in the bb region can be detected immediately by the absence of y B females (y/S-5) from the culture, since S-5 is deficient for most of the proximal heterochromatin, including the locus of bb.

Lindsley, D. L., and Edington, C. W. Failure to close the YSX.YL chromosome.

For several years we have been interested in obtaining a ring-shaped XY chromosome. To this end we have constructed a chromosome of the constitution $y^+Y^SX.Y^Ly^+$; this was done by detaching a reversed acrocentric compound-X chromosome, which had Y^Ly^+ (derived from sc^8.Y) as a second arm, with sc^8.Y:bw^+ ($=y^+Y^S$.bw^+Y^L, see Baker DIS-29). Males carrying the $y^+Y^SX.Y^Ly^+$ chromosome and no free Y were irradiated and crossed to y^2 su-w^a w^a bb/O females. Simultaneous loss of y^+ from each end

of the $y^{+}Y^{S}X.Y^{L}y^{+}$ chromosome, which results in recovery of a y male, was considered strong presumptive evidence of ring formation. Retention of both Y^{L} and Y^{S} fertility factors is required for fertility of the y males; on the basis of Baker's observations on fractionation of $sc^{8}.Y:bw^{+}$ we expected roughly 1% of these males to be fertile. To date we have recovered 155 y males, all of which have been sterile.

Makino, S., Takada, H., and Lee, J. J. Drosophilidae from Kongju in South Korea.

Several drosophilids were reported in Korea by Kikkawa and Peng, 1938; Nakayama and Okamoto, 1940; and Chung et al., 1955, 1956. A collection was attemped in Kongju and its vicinity during the period from September, 1956 to August, 1957. Twenty-four species were collected, by means of small jars baited with fermenting fruits, and with a sweep net. It was found that twelve of them were new to the fauna of Korea. They are as follows: Amiota variegata, Leucophenga quinquemaculata, Mycodrosophila basalis, Drosophila angularis, D. auraria, D. bifasciata, D. brachynephros, D. lacertosa, D. melanissima, D. nipponica, D. testacea, and D. unispina.

Mead, C. G. The effect of Bar, Enhancer of Bar, and changes in their positions on the free amino acids and peptides of D. melanogaster.

Stocks were constructed of car, B car, BB car (by unequal crossing over), En-B car, and B En-B car, co-isogenic with one another for their autosomes and their X chromosomes except for the B--En-B region. (Original material kindly provided by G. Bonnier.) The free amino acids and peptides of each genotype were analyzed by two-dimensional chromatography of twenty whole, squashed flies, five days old; and the resulting ninhydrin-positive sport were measured densitometrically. Ten chromatograms were made of each genotype, and the concentration of each spot was expressed as the mean proportion of the total free ninhydrin-positive material measured. The amino acids identified by co-chromatography were aspartic acid, glutamic acid, cystine, serine, glycine, threonine, taurine, lysine, glutamine, alpha-alanine, beta-alanine, tyrosine, proline, histidine, arginine, and methionine and/or valine and/or tryptophan, of which aspartic acid, glutamic acid, cystine, serine, glycine and taurine (measured together), threonine, alpha-alanine, beta-alanine, glutamine, and histidine and arginine (measured together) were measured quantitatively. A peptide, "pupine" (probably corresponding to Chen and Hadorn's P_2), and a front peptide were also identified, of which pupine was measured quantitatively.

The Bar position effect was demonstrated to be exhibited in terms of free amino acids by comparing the results of the analyses of B car/B car and BB car/car, which differ significantly in their proportions of pupine, aspartic acid, glutamic acid, cystine, serine, glycine and/or taurine, beta-alanine, and histidine and/or arginine. The Enhancer of Bar position effect was also demonstrated, but proved to be less extreme than the Bar position effect. This was shown by comparing the results of the analyses of En-B car/ B car and B En-B car/ car, which differ significantly with respect to serine, alpha-alanine, glutamine, and histidine and/ or arginine. It is also of interest that the presence of Enhancer of Bar results in a decrease in the proportion of threonine and alpha-alanine and an increase in the proportion of the peptide, pupine. This result suggests that pupine may possibly be a

peptide consisting largely or entirely of the two amino acids, threonine and alpha-alanine. This possibility is being tested by elution of the pupine spot and subsequent hydrolysis.

These results demonstrate that the position of Bar and Enhancer of Bar not only have an effect on eye facet number but also have an effect, although different, on the constitution of the free amino acids and peptides in the hemolymph and tissues of the adult.

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Meyer, Helen U. Induction of autosomal lethals by ultraviolet treatment of male first-instar larvae of D. melanogaster.

Though the embryonic polar cap has proved to be the most suitable stage for ultraviolet treatment of the germ cells of D. melanogaster, it is occasionally desirable to use a different phase in the life cycle. Irradiation of adult males, successfully carried out by several investigators, is limited to certain genotypes with little pigmentation. E. Altenburg (1934) irradiated 2- to 3-day-old larvae with U. V. over a period of several days, but concluded from the low yield of sex-linked lethals (.14% as contrasted with .03% in the controls) that this stage is almost as impervious to U. V. as the adult stage.

Lately we have treated first-instar larvae during the first hour after hatching; at this time they are still quite transparent, and the gonads of males are close to the dorsal surface and fairly unobstructed, whereas those of females are embedded in fat tissue. The larvae were placed in water, and held in place by slight pressure between a glass and an overlying quartz plate, through which the radiation was given.

Two series of experiments were performed, both of which utilized similar stocks and the same genetic methods to score recessive lethals in chromosome 2. In both instances, an increase of lethals was found in treated males as compared with treated females and untreated controls. Some of these lethals appeared as allelic clusters among the offspring of a given treated male, indicating that mutation occurred during early germ-cell development, the stage when radiation had been applied. The first, pilot experiment used germicidal U. V. (mainly 2537 A; surface dose, 585 ergs/mm^2), whereas the main experiment used longer-wave U. V. (mainly 2900 to 3100 A; surface dose, 112,000 ergs/mm^2). Though much less effective mutagenically, this longer ultraviolet has greater power of penetration and is less detrimental to the survival of the larvae to adulthood (50.8%). The following results were obtained:

Experiment	Treated males	Treated females	Controls, both sexes
Pilot expt. (2537 A)	9 leth./341 chroms. 2.6 ± 2.1% lethals	1/200 0.5 ± 0.5%	1/1045 0.1 ± 0.1%
Main expt. (2900-3100 A)	30 leth./1269 chroms. 2.3 ± .6% lethals	1/192 0.2 ± 0.2%	9/2306 0.4 ± 0.1%

Thus, a significant increase in the lethal rate from males was found in the main experiment; the germ cells of treated females remained unaffected, no doubt because they failed to be reached by the irradiation for reasons discussed above. Though the mutation rate obtained is comparatively low (corresponding to about 1% for sex-linked lethals), this technique of treating males is practicable in large-scale experiments.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Milani, R. Housefly genetics: linkage of kdr (knock-down resistance to DDT) with bwb and dv.

Two-point and three-point tests showed linkage between the genes dv (divergent wings), bwb (brown body), and kdr (knock-down resistance to DDT). The tests have been carried with the markers in coupling and in repulsion. Backcross tests gave the following approximate recombination ratios. Heterozygous females (consistent results in different series): bwb-dv, 40%; bwb-kdr, 49%; dv-kdr, 45%. Heterozygous males (obviously different data from two different series have not been pooled): bwb-dv, 0.00% and 2.40%; bwb-kdr, 0.42% and 1.12%; dv-kdr, 0.58% and 3.46%. The recombination for bwb and dv (syn. div) are consistent with that already published (DIS-30: 138); male recombination--varying between series--has been confirmed.

Milani, R., and Palenzona, D. Hatched larvae in the uterus of D. melanogaster.

In dissection of D. melanogaster females from old, overcrowded bottles of a strain originally collected in nature and kept in captivity for five years, it was observed that most of them were carrying in the uterus an egg at a level of development just before the emergence of the larva. In some females, however, a hatched larva was already present, moving above the chorion distended on the ventral surface of the uterus; in all cases the head of the larva was directed toward the ovaries. The larvae taken out by dissection behaved normally on fresh culture medium, without signs of suffering. Attempts to observe parturition of living larvae were unsuccessful. However, active larvae were observed a few minutes (from three to five) after the introduction of fresh food into a population cage; one was obviously wounded. Among the normally hatched chorions were others that were clearly abnormal and crumpled; counts of chorions and larvae showed correspondence between the number of larvae and the number of normal-looking chorions. This suggested retention (or resorption) of larvae hatched in the uterus. In one single case a female clearly showed difficulty in laying what proved to be an egg with a partially hatched dead larva. This female made short runs, and stopped to perform unsuccessful oviposition movements alternated with lifting and perhaps repulsive turning of the abdomen. During these antics, which lasted some five minutes, it ate "nervously." After parturition it moved away with a sudden run, and behaved normally thereafter.

Miller, D. D. Variation of the Y chromosome in D. athabasca.

As originally described (Sturtevant and Dobzhansky, 1936), D. athabasca has a J-shaped Y chromosome. This has been verified in recent strains of both eastern and western localities. However, a strain from Ontario (Algonquin Park) was found to have a small V-shaped Y (smaller than the large V-shaped

X). Two other strains, one from northern Michigan (Iron Mountain) and one from New York (Cold Spring Harbor), were found to have a large V-shaped Y (similar to the X) associated with the rod-shaped autosome, which was present only once in males, thus indicating a translocation between this autosome and the Y. One of these aberrant strains (C.S.H.) has been used extensively in crosses with a Wyoming strain having a normal J-shaped Y, and these crosses have yielded fertile hybrids; hence, the unusual sex-chromosome relationship of the Michigan and New York strains does not indicate a new species.

Miyoshi, Y. Physiological significance of yellow pigment. The yellow pigment extracted from the mutant strain sepia of D. melanogaster, which does not seem to occur in recognizable amounts in the wild strain Oregon R-S, is very much like flavin in its chemical composition. The pigment, however, is not precipitated by NaCl, whereas flavin makes an insoluble compound with it. This fact suggested that the yellow pigment might play some important role in lieu of flavin in the presence of high concentrations of NaCl in the body of the mutant. Therefore, these two strains were tested for differences in susceptibility to excess amounts of NaCl. The eggs were allowed to hatch on, and fed with, Carpenter's semisynthetic medium (DIS-26, p. 132) containing NaCl in concentrations of 0.5 M, 1.0 M, and 2.0 M. The cultures were kept at 25° ± 1° C, and survivors were counted at each stage of development. The results obtained were as follows:

Conc. of NaCl in medium (M)	Strain	No. of Eggs	Unhatched %	2nd instar %	3rd instar %	Pupae %	Emerged No.	Emerged %
0 (control)	se	500	5.6	72.8	65.0	59.8	♂125 ♀132	51.4
	+	500	7.6	88.4	77.8	75.6	♂172 ♀196	73.6
0.5	se	500	6.0	57.6	43.6	39.6	♂ 90 ♀ 71	32.1
	+	500	10.4	51.2	37.6	36.0	♂ 81 ♀ 84	33.0
1.0	se	450	10.4	29.1	22.2	21.3	♂ 33 ♀ 42	16.7
	+	500	10.6	2.2	2.0	2.0	♂ 8 ♀ 2	2.0
2.0	se	400	9.0	9.5	0.75	0.75	♂ 1 ♀ 1	0.5
	+	500	9.8	0	0	0	♂ 0 ♀ 0	0

The data show significant differences between the strains when the NaCl concentrations were 1.0 M and 2.0 M. These were chiefly due to differences in mortality in the first and second instars. The results clearly indicate that se is more resistant than the wild stock to a high concentration of NaCl.

Momma, E. The behavior of the paranucleus in the spermioteleosis of D. lacertosa.

The morphological changes leading to the formation of the paranucleus during spermatogenesis were reported in a few words by the author in DIS-30. At first the mitochondrial masses are two in number, and structureless. In the early spermatid stage they come together into a single body to form the nebenkern, which is considerably larger than the nucleus. It shows a rapid increase in volume to about ten or more times that of the nucleus, and assumes a lamellar, concentric structure. Presently the nebenkern undergoes a change in appearance; the lamellae seem to decrease in number and to increase in thickness by coming together at several points. The lamellae seem to be double in structure, dividing into two threads. Sometimes, several deeply stained granules become observable outside the lamellae, or within the interlamellar matrix. Meanwhile, the nebenkern is divided into two parts, which form two progressively elongating halves. The lamellae decrease still more in number, though their profile is not clear. First, each half of the nebenkern elongates in a curve within the spermatid. Then both halves elongate further as the spermatid itself elongates. A well-stained fine thread appears in the cell body. Deeply staining minute beadlike bodies appear along the thread in the late spermatid stage.

Morita, T. Purine catabolism in D. melanogaster.

It was demonstrated by Hadorn et al. that the eye-color mutant rosy^2 (ry^2) does not contain isoxanthopterin. Substances with fluorescence or absorption at about 260 mμ have been identified by means of chromatographs and absorption spectra, in ry and Oregon-R flies.

It was found that ry did not contain isoxanthopterin at any developmental stage, but after pupation it contained more AHP (2-amino-4-hydroxypteridine) than the wild-type strain. Uric acid was not contained at any developmental stage in ry, but ry after pupation contained hypoxanthine instead of uric acid. Hypoxanthine was not detected in the wild type by the chromatographic method. The results are shown in the table.

	Oregon-R			ry		
	Larva	Pupa	Adult	Larva	Pupa	Adult
Isoxanthopterin	±	++	++	-	-	-
AHP	±	+	+	±	++	+
Uric acid	±	++	+	-	-	-
Hypoxanthine	±	±	±	±	++	+

In a double mutant se ry, which showed the se phenotype, the same result was demonstrated as in ry. From these results, it seems that in wild-type D. melanogaster AHP acts as an intermediary in isoxanthopterin formation. Although xanthine was not demonstrated in ry by the chromatographic method, in the case of the wild type uric acid could be produced along the general pathway according to the following scheme: hypoxanthine ⟶ xanthine ⟶ uric acid. It seems that the activity of xanthine oxidase is deficient in ry.

Moriwaki, D., and Nakajima, Y. Reciprocal effects on heterosis in D. ananassae.

In D. ananassae, the gene arrangements, A and B, resulting from the inversion InIIL can be paired in four genotypes: AA, BB, AB, and BA. Comparative studies of rate of development in these four types show the following relationship, AB > BA = AA > BB. It is clear that the two heterozygotes produced by reciprocal matings differ significantly in rate of development; that is, those derived from A mothers (AB) seem to develop faster than those from B mothers (BA), the homozygote AA being superior to the homozygote BB in rate. More rapid development resulted from a mating in which the female had the higher rate of development, at least under optimal conditions, contrary to results obtained with D. persimilis by Spiess.

Later, in a comparison of the development of offspring from AB mothers with those from BA mothers, it was found that the former developed faster than the latter regardless of the source of sperm. Consequently it can be said that the cytoplasmic character which produced these differences in reciprocal crosses is effective to at least the next generation. In spite of the cytoplasmic effect, differences in genotypes could be recognized in the two kinds of back-cross offspring of each group. However, it is not yet known whether these two effects are additive.

Mourad, A. M., Mallah, G. S., and Tantawy, A. O. Frequency of heterozygous inversions in natural populations of D. melanogaster and D. simulans.

D. melanogaster and D. simulans, collected from eight different geographical regions, were examined cytologically for frequency of inversions on the second and third chromosomes. (For the different localities mentioned, see research note by Dobzhansky, Mallah, Tantawy, and Mourad in this issue.) The results obtained for D. melanogaster from five of these regions are given in the table.

Locality	No. of ♀♀	% of inversions in each chromosome			
		2nd chromosome		3rd chromosome	
		L	R	L	R
University Farm	110	20.00	4.55	---	7.27
Fayuom	54	3.70	1.85	---	29.62
Mehalla El Kobera	111	20.72	---	0.90	5.40
Wadi El Natroon	94	47.87	---	8.51	13.82
Lebanon	66	7.57	---	---	3.03

The collection from Abou-Sir involved mostly D. simulans and contained only three females of D. melanogaster. Two of these females were found to have inversions on the second chromosome (R); the third had an inversion on the third chromosome (R). From Beni-Swef, the flies collected were mainly D. simulans, with only nine females of D. melanogaster. Of these nine females, three carried inversions, one each, on the third chromosome (L). D. melanogaster captured in Kom-Ombo (N = 13) did not show any inversions.

The results presented in the table show that most of the inversions were found on the second (left) and third (right) chromosomes. Flies from Wadi-El-Natroon showed the highest percentage of inversions on the second chromosome,

and those from Fayuom the highest percentage of inversions on the third chromosome.

No inversions have been found so far in any of the D. simulans collected from these localities.

Experiments are still in progress, and more populations will be investigated as well as hybrids between them.

Muller, H. J. Mutation studies of chromosome 3 simplified by the "sifter-3" method.

The chief bottleneck in the detection of recessive autosomal mutations at non-specific loci, which has limited the number of chromosomes that can be tested, has been the necessity of inbreeding with one another just those F_2 individuals of each F_1-F_2 culture that have inherited the same specified autosome of the F_1 individual being tested. This operation ordinarily requires the virtually simultaneous collection of virgin females of the specified type from each of the numerous F_1-F_2 cultures. For chromosome 2, several schemes to overcome this difficulty have been devised by the writer (Anat. Rec. 24: 419, 1923; Genetics 13: 279-357, 1928; DIS-25: 117-118, 1951; DIS-27: 104-105, 1953), but for chromosome 3 only one scheme (DIS-29: 147-149, 1955). The scheme now being offered for chromosome 3, called "sifter-3," has the following advantages over the previous one: its operation is simpler; it requires fewer stocks; it kills, instead of sterilizing, all F_2 males of the undersired types; it gives rise only to the types of F_3 desired, and requires no selection among the latter and no collection of virgin F_3.

The main principle involved in the operation of the sifter-3 method is that of the lethality of hyperploid individuals that have four doses (tetraploidy) of the region of chromosome 2 extending from about 59C to 60E. This is the region common to the Pale transposition (59A1-60E1) and the Y:bw$^+$ insertion (about 59C-60E5±4). At the same time, hyperploids having three doses of this region (or of the slightly longer one of either of the duplications just mentioned) are viable and fertile. It is arranged that all F_2 males of the undesired kind are either tetraploid for this region or killed by being homozygous for a lethal in chromosome 3. Thus the F_2 females can have mated only with males of the desired kind. The F_2 flies must be etherized, however, so that the desired type of nonvirgin F_2 females can be picked out for making up the F_2-F_3 cultures.

The scheme requires that the F_1 males containing a chromosome 3 that is to be tested for recessive lethal and/or visible mutations have a homologous chromosome 3 provided with inversions and a dominant marker (the combination ru h D InsCXF e^s being in most cases the most useful for this purpose), and also have a Y chromosome of type Y:bw$^+$, carrying the 59C-60E section of 3 inserted in its long arm. The P_1 male should have had its two chromosomes 3 (if both of them are to be tested) differentiated in respect to one or more of the recessive markers (such as ru, h, or e^s) carried by the dominantly marked chromosome of the F_1, so that the F_1 males are distinguishable with regard to which chromosome 3 of the P_1 male being tested they contain. Thus, the composition of a given F_1 male may be Y:bw$^+$; h ri/ru h D InsCXF e^s ♂, and that of a brother may be Y:bw$^+$; ve bv/ru h D InsCXF e^s ♂. Such F_1 males can have been derived from P_1 males of composition Y:bw$^+$; h ri/ve bv crossed by virgin females of composition e P^i/ru h D InsCXF e^s. P^i designates section 59A1-60E1 (of the Pale transposition) inserted into chromosome 3. The F_1 males (see above) are individually backcrossed to virgin females of this same

type. In the F_2 generation, all males getting the e P^i-containing chromosome 3 are killed by hyperploidy, since they also have Y:bw^+ that contains section 59C-60E (except for any males which because of nondisjunction lack a Y and are therefore sterile). Of the F_2 males getting the D-containing chromosome 3 of their mother, those also getting this chromosome from their father (the F_1 male) will be homozygous for lethals, while only those getting the chromosome to be tested can live; these are of the desired type for breeding. The females of the same chromosome-3 type as the males are picked out for mating with them.

A sample pedigree illustrating the above is as follows:

P_1 Y:bw^+; h ri/ve bv ♂ x e P^i/ru h D InsCXF e^s ♀
F_1 Y:bw^+; h ri or ve bv/ru h D InsCXF e^s ♂ x ♀ like P_1 ♀
F_2 All viable males are like father (F_1 ♂); females (nonvirgin) having chromosomes 3 like that of F_1 ♂ are picked out for breeding.
In F_3 look for presence of non-Dichaete and check their phenotypes.
(The F_2-F_3 cultures already constitute balanced stocks of whatever chromosome-3 lethal or other mutant it may later be desired to save.)

A minor defect in the scheme arises from the fact that if any of the females to which the P_1 or F_1 males are mated should contain a normal Y (or if the P_1 males should contain one), one or a few viable fertile F_2 males containing the e P^i chromosome may be produced by nondisjunction. They would however be recognizable by being either non-Dichaete or Dichaete ebony, and cultures containing such males may be discarded. Even if they were used, mutations would still be recognizable among the F_3 by looking for (or at) the non-Dichaete non-ebony flies. In any case this situation would not cause nonlethals to be s cored as lethals (a much more serious error than the converse one). A stock for furnishing the e P^i-containing females is now under construction in which this source of difficulty will be obviated by having the males supplied with a Y:bw^+ instead of a Y^+ chromosome; females with the Y:bw^+ in addition to their e P^i would be inviable.

The P_1 males of the above scheme can be obtained by crossing two "P_0" stocks having the respective chromosomes 3 of the P_1 and having (at least in the P_0 stock that furnishes the male in the P_0 cross) a Y:bw^+ instead of ordinary Y. However, the chromosomes 3 of flies of any desired stock (or from nature) can be tested by crossing females containing them to males having Y:bw^+ and ru h D InsCXF e^s, or by crossing males containing them to females having Y:bw^+, attached X's, and ru h D InsCXF e^s. These crosses at once provide F_1 males of the type shown in the preceding scheme, but they can if preferred be used as "P_1" if the mutation frequency of their own generation is to be ascertained. A stock that is being made up for this purpose has the composition Y:bw^+/X^+& X.X ("snoct"); ru h D InsCXF e^s/Mé, Ins ri Sb^1.

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Muller, H. J., and Edmondson, M. Transposition of entire 4-euchromatin into a fully functional Y.

A translocation between the Y and the fourth chromosome, with functional male-fertility genes, was found by Edmondson in 1946, in x-rayed material, and has been carried among the Indiana stocks ever since (see DIS-26, p. 20, 1946, et seq.). Recent genetic analyses by Muller have shown that the whole

of the euchromatic portion of 4 has been inserted into the Y, leaving both chromosomes fully functional without any other parts of 4 or Y being required. Thus the case fits the definition of "transposition," a deletion from one chromosome into a nonhomologous chromosome, but it is unusual in being a whole-arm transposition.

Muller has constructed a stock of it, denoted simply by Tp4:Y, or still more simply by Y:4, in which all females as well as males contain it in diploid dose and are free from all other Y and 4 chromosomes and chromosome parts, and another stock, in which the Y^L arm of Novitski's Y^S.X InEN y.Y^L sc^8 y^+ has been exchanged for the portion of the Y:4 chromosome that includes Y^L and 4 but not Y^S. We are not yet sure whether 4 lies on the same side of the centromere as Y^L, or whether the centromere of Y:4 was derived from Y or from 4. However, four breaks would have been required if the centromere were from 4, otherwise only three.

That the inserted 4 affects the recombinational properties of this Y is indicated by the fact that in a count of 12,187 offspring from females containing ordinary attached X chromosomes homozygous for yellow, besides a Y:4 and two free 4's homozygous for ci ey^R, crossed to males with a Y:4, a normal X, and likewise two free 4's with ci ey^R, not a single case of "detachment" of the attached X's was found. Ordinarily these attached X's in company with a normal Y would have given 8 or more spontaneous detachments, by exchange between the X.X and the normal Y. However, the above-mentioned case of attachment of Y^L:4 to Novitski's "X.Y" did involve an exchange of much the same kind as is in question here.

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Muller, H. J., and Oster, I. I. Suppressor action effective with a subgene deficiency of a normally duplicated locus.

An X-ray-induced forked of mosaic expression (Muller, DIS-20: 88, 1946) was found to have involved a simultaneous change in two positions (Muller, DIS-21: 71, 1947): (1) a recessive mutation to a moderately expressed forked, "f^x," which had arisen in the position usual for this character and which showed ordinary crossing-over relations, and (2) a gene having a dominant suppressor-like action on forked, of mosaic expression, located in the heterochromatin of the X near the centromere; addition of a Y chromosome increased the normalization of the forked character caused by the heterochromatically placed mutant. It was inferred from this simultaneous origin of a complementary hypomorph and hypermorph in different positions that they did not represent a mere coincidence but that, as in the case of the Pale transposition for which Altenburg in 1918 made a similar inference on the same grounds, a region had been removed from one position, here that of f^x, and inserted into the other position (a change designated as a "shift" when the deficiency is in the same chromosome as the insertion). The gene in the heterochromatic location was therefore designated "f^{+ih}" (i for insertion and h for its heterochromatic position), and both its mosaic expression and the normalizing effect of extra heterochromatin upon its action were in accord with these effects in other cases of variegation.

It was thereby implied, although not expressly stated, that f^x was really a deficiency, of the piece represented in the f^{+ih} duplication. More-

over, since crossovers having f^x without f^{+ih} were found to have a viability and fertility usual for forked, along with only a moderate expression of the forked character, unlike what was known to be true for chromosomes showing a deficiency of the entire forked region, it was self-understood that the f^x deficiency involved only a portion of the region functionally concerned with forked. That is, this region must normally be made up of two or more subloci or subgenes, not all of which had been shifted to the f^{+ih} position. Direct proof of the compoundness of the forked region, by means of experiments showing that the different forked alleles tested fall into two groups, the members of either of which show crossing over with the members of the other group, was later provided by M. M. Green (P.N.A.S. 41: 375, 1955, and P.N.A.S. 42: 73, 1956).

Valencia (reported by Muller, 1946, ibid.) had found that the f^x deficiency is too small to be seen in salivary chromosomes, as is only to be expected of a subgene deficiency. Further evidence of the deficiency nature of f^x was obtained by Oster (reported by Muller and Oster, Advances in Radiobiology: 407, 1957), in the finding of no case of spontaneous or induced back mutation of f^x in counts that would have given an expectation of 6 cases if forked-1 had been used. This does not, however, imply that f^x is unable to give back mutations, as by means of duplication of the remaining region; but most back mutations of forked are not duplications, as shown by the fact that their frequency in ring X's is about as high as elsewhere (see Muller and Oster, ibid.). It may be concluded that in this region hypermorphic mutations of normal subgenes are either less frequent or less effective than those of mutant subgenes.

It might seem natural to assume that because f^x is a deficiency it cannot be suppressed by a modifier, such as Whittinghill's su-f, especially since Green (ibid.) found that only about half of the spontaneously arisen forked alleles tested by him (including none known to belong to his second locus-group) and none of the X-ray-induced ones (of either locus-group) were suppressible by su-f. In our laboratory Sara Frye has recently obtained a similar series of results on spontaneous and induced forked alleles, not yet published. However, f^x has been found by us to be definitely suppressible by su-f. We may conclude from this not only that some X-ray-induced forkeds are suppressible but also that suppressibility does not constitute evidence against deficiency. Conversely, the unsuppressibility of the previously tested X-ray-induced forkeds cannot constitute evidence that they <u>are</u> deficiencies, as might otherwise have been suspected.

This result calls for a revision of thinking concerning suppressor action. It seems to have been tacitly assumed by some students of the subject that when a forked or other mutant is suppressible, the suppressor is somehow stepping up the effectiveness of the reactions initiated by that mutant gene or subgene itself. This is impossible, however, when the given gene or subgene is actually absent, as in the case of f^x. Here, then, it is the effectiveness of the reactions initiated by the remaining subgene(s) that is stepped up, so that they are able to compensate for the deficiency. If the same general principle holds also in the supression of nondeficient mutants, such as forked-1 and forked-5, the action of the suppressor here may really be on the sublocus that adjoins them, the one that remained normal, rather than on the mutant sublocus itself, that we had considered to be suppressed. The suppression would consist in an enhancement of the unmutated sublocus, causing it to react, in effect, hypermorphically. Thus when forked-1 was suppressed it might be the sublocus of forked-3 that was enhanced. However, it is also possible, in the case of nondeficient mutants,

that the gene-initiated reactions of the different subloci may merge at a stage preceding that at which the "suppressor" action is exerted, so that it would be improper to refer the suppressor effect to a reaction on the product of any given sublocus.

We have used the term subgene advisedly in this case, since the above-noted evidence indicates that the forked region of the normal X chromosome is compound in the sense of consisting of a number of separable parts of similar structure and function just as does the scute region of the X (viz., at least achaete and scute) and the male-fertility regions of the Y. Only in these three cases has this conclusion, to our knowledge, been well supported. The support involves evidence, in each case, indicating that when a break occurs in the given region, followed by a linear separation of its parts, both parts continue to some extent to perform the function in question.

In cases exhibiting only the more usual evidence of pseudoallelism based on crossing over, on the other hand, it is always possible to postulate, and often there is direct ground (based on the difference between cis and trans compounds) for concluding, that the similarity of action of mutants occupying nearby positions has been caused by a "position effect," a reaction occurring adequately only within a minute distance between one gene or gene product and a neighboring gene or gene product. When this is the case, however, it is usually a sufficient explanation in itself, without the postulation of a similarity in actual structure and mode of action of the cooperating genetic entities. Thus, in these very cases we lack grounds for inferring that the given region is of duplicational nature or origin.

In the case of forked, as in that of scute and the Y, it is the structural change that gives us evidence that each part of the region, even when separated from the other, can to some extent perform the function of the other, inasmuch as (1) the remainder present in its normal location in the f^x chromosome does not have the extreme forked effect shown by a full-fledged deficiency and (2) the shifted material (when the incapacitating effect of the adjoining heterochromatin is counteracted by an extra Y) also is able to act in bristle normalization. In other words, both parts have some "anti-forked" or bristle-straightening effect that is not dependent on a positional reaction and they therefore carry on such similar functions as to lead us to conclude that they were derived from a common ancestral gene by its linear duplication.

A less likely interpretation of the insertion, f^{+ih}, is that it involved a larger piece than the deficiency, f^x, but if this were true this inserted piece would have had to be contributed by the sister chromatid to that in which the deficiency f^x occurred, and that chromatid would have sustained a larger deficiency than f^x. The complicated nature of this interpretation reduces its probability; but if this process had occurred the case would not illustrate the similar functioning of different subgenes, since the inserted piece might contain the entire region, and the evidence that the neighboring loci were products of a duplication occurring in the past evolution would thereby disappear.

If we accept, at least as a working hypothesis, the interpretation that f^x and f^{+ih} are complementary, a curious paradox is encountered in attempting to bring this case into line with Green's finding that the loci (or subloci) dealt with by him fail to produce normal bristles except when there is a chromosome having both or all unmutated loci (or subloci) close together, that is, in cis arrangement. For in our case (at least with the aid of an

extra Y) the parts even when separated, as they are in the f^{x} f^{+ih} chromosome, are able to give rise to a normal bristle phenotype. Evidently, the parts are not just the same ones as those studied by Green, or the mode of reaction of one or more parts has been altered by their mutation or positional change.

We should be glad to have anyone who wishes to do so conduct further cytological study on this material.

(The more recent work here reported has been supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT(11-1)-195.)

Muller, H. J., and Schalet, A.
Further improvements in the "Maxy" stock for detection of specific-locus mutations.

As noted in the earlier description of this stock (Muller, DIS-29: 146, 1955), the X of the male has the moderate-sized inversions In49 and B^{M1}. It also has the minute inversion that goes with the combination 1J1 sc^{J1} (mistyped sc^{S1} in DIS-29) and was provided with the marker miniature wings (m). The duplication of the left end of the X designated as $1J1^{+}$, attached to the long arm of the Y to give the chromosome designated as $1J1^{+}$.Y, allows the males to live despite the presence of 1J1 in their X, but it does not cover the scute or yellow loci (Muller, DIS-28: 140, 1954).

Unfortunately, both sc^{J1} (like most scutes) and m reduce the viability and fertility of the males. Moreover, sc^{J1} interferes with the detection of bristle mutants and m with that of wing mutants. These difficulties have now been overcome.

In place of sc^{J1} in the X chromosome of the male we now utilize $sc^{J1(+)}$, which was found by Schalet as a spontaneous back mutation of sc^{J1} that results in a virtually normal phenotype of bristles and good viability but does not prevent the lethal effect of 1J1. In place of m we now use oc, which does not interfere with the detection of any of the genes in question, and has good viability and fertility in the male, so that approximately 45% of the flies in these cultures are males. In addition, oc has the advantage of sterilizing all those females which, having acquired a $1J1^{+}$.Y through nondisjunction, are viable even though homozygous for the 1J1-containing X chromosome. The Bar-M1 region of this chromosome contains a nonlethal moderate bobbed allele.

The insertion of oc into the In49 of this chromosome was carried out by Muller, by utilization of heterozygous inversions in chromosomes 2 and 3 simultaneously (those associated with Oster's Curly chromosome and with the Dichaete CXF complex) to step up crossing over between the X's. This insertion of oc from the X without In49 into that with In49 required double crossing over in which both points of crossing over were within the limits of this inversion. One case occurred among about 5000 offspring that had received oc. The In49 oc combination will also be useful in other connections, as for instance in our derived stock of the composition sc^{S1} B In49 sn^{x2} oc ptg sc^{8}. As usual, the oc within In49 has the nearby ptg along with it, but this has no useful function here.

As a further improvement, Schalet has obtained a representative of the multiple-recessive X chromosome of the "Maxy" stock (that carrying the specific-locus markers) which has, by spontaneous mutation, acquired a lethal.

This lies between the loci of sc and w. It prevents the appearance, even as larvae, of the multiple-recessive males, which, being unwanted, were wasteful of breeding space and scoring time, and sometimes bred. It also kills so effectively that no gynanders manifesting this chromosome have yet appeared, although in the nonlethal stocks the same number of flies had shown a noticeable frequency (1/7500 ♀) of them, resulting in mutant-like individuals. Finally, the multiple-recessive chromosome, which had been found to have lost rb in the process of inserting ec, has been repaired so that it now contains both these genes.

The stock is now constituted as follows in odd generations: $1J1^{+}.Y/1J1\ sc^{J1(+)}$ In49 ptg oc B^{M1},In ♂ and $1J1\ sc^{J1(+)}$ In49 v ptg oc B^{M1}, In/ $y^{Si}\ sc^{Si}$,In car odsy f g^{2} dy v ras^{2} sn^{3} ct^{6} cm rb ec w pn sc^{8} ♀. In even generations the sc^{J1}-containing chromosomes, having v+ and v, respectively, are interchanged between the male and female, with a resultant crisscrossing of the vermilion phenotype, as a check against the rare production of exceptions through the occurrence of nondisjunction in the mother. Nondisjunction in the father produces daughters with a $1J1^{+}.Y$, and these in the next generation produce many homozygous oc females and a few male and female exceptions with regard to vermilion; cultures showing these characteristics are not used for further breeding. It will be seen that in this scheme only one type of female and one type of male is regularly produced in any given generation, so that attention may be concentrated on finding the mutants. Moreover, the nonvirgin females can be bred individually, like ClB females, for ascertainment of the frequency of lethals in their sc^{J1}-containing X; a female with a lethal produces no sons.

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Murray, C. L., and Lewis, H.

Studies of the effect of varying concentrations of salt on recombination in D. melanogaster.

The suggestion has been put forth by several workers in recent years that chromosomes are composed of particulate units linked through divalent cations and that electrostatic attraction plays an important role in chromosome stability. This suggestion has come from experiments in which excess or deficient amounts of divalent cations are correlated with altered recombination frequency. Altering the chemical environment of chromosomes by varying ionic strength may permit the detection of some clue as to the importance of electrostatic forces in chromosome stability. The experiments reported in this note were performed to detect such clues. This was a pilot study, and no attempt was made to determine the extent of alteration of ionic strength in the cells of the various tissues.

Larvae heterozygous at the y ec ct v w y f loci were bathed in NaCl solutions of various concentrations for 24 hours during the last day of the third-instar stage. It has been shown that treatment by immersing larvae in test solutions is as effective as injecting the test solution into the larvae. Both hypo- and hypertonic salt solutions were used as test solutions (0.0, 0.5, 0.75, 1.0, and 1.2% NaCl). Control groups were untreated flies. All work was carried out at 24±1° C. If chromosomal instability was induced by this treatment it could be reflected as disturbed development of imaginal tissues or altered recombination frequency among the offspring of test-crossed treated females. Neither of these effects was observed. Nearly all the treated flies pupated and subsequently eclosed into normally developed adults. The recombination frequencies in the segments of the X chromosome

tested are not significantly different between any of the treated groups and the controls. There are a number of possible explanations of why the treatment does not affect the frequency of recombination, the most likely being that the sodium ions do not penetrate into the cells of the gonads.

Nawa, S., Taira, T., and Oshima, C. Nonenzymatic conversion of the yellow pigment found in D. melanogaster. As a result of incubation of the yellow pigment obtained from se with methylene blue at near neutrality, AHP (2-amino-4-hydroxypteridine) was produced. This result was confirmed by means of two-dimensional paper chromatography. It is concluded that DPN (diphosphopyridine nucleotide) cannot serve as a hydrogen acceptor. The other pterins found in D. melanogaster, such as 2-amino-4-hydroxypteridine-6-carboxylic acid and 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine, were not affected by incubation with methylene blue.

Nawa, S., Taira, T., and Oshima, C. Pterin dehydrogenase and its coenzyme in D. melanogaster. An enzyme which was found in our laboratory in a homogenate of D. melanogaster can catalyze the oxidation of AHP (2-amino-4-hydroxypteridine) to isoxanthopterin, and it may be capable of converting xanthine into uric acid. The pH optimum of this enzyme is in the neighborhood of 8.5. The enzymatic oxidation of AHP to isoxanthopterin could not be catalyzed without a cofactor such as methylene blue or DPN (diphosphopyridine nucleotide), when a dialyzed preparation was used. Because of its nature this enzyme was named "pterin dehydrogenase." When an undialyzed preparation was used, however, the enzymatic oxidation progressed at an appropriate rate without the addition of methylene blue or DPN.

Our experiments showed that DPN was a more effective electron acceptor than methylene blue. Therefore it seems that DPN may be a more useful coenzyme of this enzyme in vivo.

From our evidence it appears that an enzyme obtained from mild and mammalian liver immediately catalyzed the oxidation of both pterin and xanthine by molecular oxygen. Further, a preparation from chicken liver reacted only very slowly with molecular oxygen as compared with either methylene blue or DPN. In this case, however, the ability of DPN as an electron acceptor was approximately 10 per cent that of methylene blue.

In another experiment, an enzyme obtained from the silkworm in our laboratory showed a similarity to that from D. melanogaster. The mode of action of this enzyme in insects, therefore, differs functionally from that in mammals or birds.

Ogita, Z. Resistance to phenylthiocarbamide (PTC) and phenylcarbamide (PC) in D. melanogaster. PTC is well known not only as an inhibitor of melanin formation but also as a substance used in diagnosing "tasters" and "nontasters," the trait being inherited as a Mendelian recessive. Effects of PTC and PC on the emergence of flies were investigated by measuring percentages of flies emerging on a dry yeast medium (agar 2 g, dry yeast powder 3 g, sugar 4 g, in 100 ml water) containing PTC in concentrations of 0.5 mM, 1.0 mM, and 3.0 mM, and PC in concentrations of 5.0 mM, 10.0 mM, 50.0 mM, 70.0 mM, and 100 mM.

The order of tolerance to PTC was as follows: y ÷ y w f ÷ w > Oregon (iso) ÷ e^{ll}(coiso) ÷ B e^{ll}(coiso) > w^e > Canton-S > e^{ll} ÷ Oregon > bw ÷ Hikone-R > Oregon-R-(I) ÷ y Oregon-R-(I) ÷ v Oregon-R-(I) ÷ v. Emergence of Oregon, bw, Hikone-R, Oregon-R-(I), y Oregon-R-(I), v Oregon-R-(I), and v was inhibited markedly by the addition of 2.0--3.0 mM PTC to the dry yeast medium. On the other hand, y, y w f, w, Oregon(iso), e^{ll}(coiso), and B e^{ll}(coiso) were resistant to 5.0 mM PTC.

The order of tolerance to PC was as follows: Hikone-R > e^{ll}(coiso) ÷ Oregon(iso) ÷ Canton-S ÷ y w f ÷ w. Emergence of e^{ll}(coiso), Oregon(iso), Canton-S, y w f, w, etc. was inhibited markedly by the addition of 50.0 mM PC to the medium, whereas the Hikone-R strain could resist up to 100.0 mM PC.

It is of interest that the Hikone-R strain, which shows "cross resistance" to various insecticides such as DDT and BHC, is less resistant to PTC than the Canton-S strain (susceptible to DDT, BHC). On the contrary, the relation to these strains to PC seems to be reversed. Therefore, the mechanism of inheritance for resistance to PTC seems to be different from that to insecticides like DDT, BHC, and parathion.

Oksala, T. A. The mechanism of secondary nondisjunction of X chromosomes and autosomes.

The following types of females were tested with respect to frequency of secondary nondisjunction of the X chromosomes:

	Mother	Percentage of sec. nondisjunction
1.	w/w/Y	7.2%
2.	w/w^{m4}/Y	16.2%
3.	w^{m4}/w^{m4}/Y	17.3%
4.	w^{m4}/w^{m4}/Y; Cy/+	10.0%
5.	w^{m4}/w^{m4}/Y; T(2;3)rn/+	8.1%
6.	w^{m4}/w^{m4}/Y ; Cy/T(2;3)rn	2.3%

In case no. 6 the disjunctional progeny exhibited the following peculiarity: in the + fraction, about 90% of the flies possessed a Y chromosome, whereas in the Cy fraction only about 10% had a Y chromosome.

All the results listed above can be explained on the assumption that bouquet orientation occurs in the early meiotic stages. The distal ends of all the chromosome arms and the heteropycnotic Y chromosome are situated close to each other at the base of the bouquet, where the pairing begins, whereas the centromeric regions are raised to the top of the bouquet as a result of the pairing process. The interpretation is as follows.

When the X chromosomes are heterozygous for an inversion (case 2) they pair with a loop. Accordingly, they do not rise to the top but remain at the base of the bouquet. The Y chromosome situated there now has a better opportunity to pair with the heterochromatic blocks of both X chromosomes than is normally the case. The result is a high percentage of nondisjunction as compared with the normal situation (case 1). The fact that X chromosomes remain at the base of the bouquet is thus the primary reason for the higher percentage of nondisjunction.

Cooper's studies (Proc. Natl. Acad. Sci. 34: 179-187, 1948) have shown that the more X chromosomes differ in structure, resulting in slower and more

difficult pairing, the higher is the percentage of nondisjunction.

Case 3 shows that even when there is inversion homozygosity of the X chromosomes the percentage of nondisjunction may be exceptionally high, provided that the inversion is of the type of In(1)w^{m4}, in which the heterochromatic block of the X has broken and a considerable portion of it has been transferred to the distal end of the X. Situated thus, it remains at the base of the bouquet, and this heterochromatin of both homologues readily pairs with the Y chromosome.

The two inversions in the second chromosome of the Cy stock significantly decrease the rate of nondisjunction of the X chromosomes, as has been known since the studies of Sturtevant (Carnegie Inst. Wash. Year Book 43: 164-165, 1949). Since the two second chromosomes, which pair by making two inversion loops, remain at the base of the bouquet, their heterochromatin has an opportunity to pair with the Y chromosome. Since as a consequence the X chromosomes, in many cases, remain free and segregate normally, the result is a decrease in the percentage of their nondisjunction. The pairing of the Y with the second chromosomes obviously fairly often leads to (secondary) nondisjunction of the latter. This can be concluded from the fact that flies without a Y produce during the same period more than twice the progeny produced by flies with a Y. Obviously the latter, owing to the nondisjunction just mentioned, lay a large number of two-II and no-II eggs, which naturally give rise to lethal zygotes. Since these inviable zygotes are all disjunctional with respect to the X chromosomes, the actual percentage of nondisjunction is still lower than the 10% obtained in the experiment. The inversions of the third chromosome have a corresponding effect, but the data from these experiments are as yet insufficient.

Case 5 proves that (autosomal) translocation heterozygosity also decreases the percentage of secondary nondisjunction of the X chromosomes. T(2;3)rn is practically a whole-arm transfer between II and III (cf. Carlson, DIS-30: 109, 1956). Pairing in the heterozygote rotund thus gives a group of four, with an exchange of partners near the centromere of each chromosome. In such a configuration the pairing is slower than normal, since each arm pair forms an obstacle to the pairing of neighboring chromosome arm pairs (cf. Oksala, Hereditas 38: 449-480, 1952). Obviously, the pairing often remains incomplete, since in the rotund heterozygote the crossing-over percentages for all the arm pairs are much lower than normal. Owing to this slow and incomplete pairing, the proximal regions of the chromosomes are delayed longer than usual at the base of the bouquet, where their heterochromatic blocks offer an attractive pairing partner for the Y. Thus the X chromosomes frequently remain free and their nondisjunction rate is decreased.

Case 6 displays the strong combined effect of autosomal inversion and translocation heterozygosity. The pairing pattern of the autosomes is largely the same as in the foregoing case. The nontranslocated second chromosome, however, obviously pairs only with the distal parts of its arms, since pairing with loops is practically impossible in a group of four. It is the heterochromatic block of the Cy chromosome which, having remained at the base of the bouquet, pairs most easily (in 90% of the cases) with the Y and segregates from it, thus going to the same pole with the two translocated chromosomes.

Oksala, T. A. Pairing pattern of the chromosomes in female meiosis in Drosophila and the "interchromosomal effect on crossing over."

The experimental results reported in the preceding note make it appear highly probable that in the early stages of meiosis the Y chromosome, when present in the female, is able to pair with the heterochromatic blocks not only of the X but also of the two large autosomes. Consequently, it can further be assumed that a general pairing affinity exists among the heterochromatic regions of all, even heterologous, chromosomes. In the same report it was also shown that many of the phenomena connected with secondary nondisjunction of the chromosomes are explicable on the assumption that a bouquet orientation prevails during the pairing process of the chromosomes. On these ideas is based the following hypothesis, which has been advanced to explain a number of important findings concerned with the "interchromosomal effects on crossing over."

If all the chromosomes are structurally of the wild type, all the proximal heterochromatic parts (6 blocks in all) are situated at the top of the bouquet. There each heterochromatic block has the opportunity to pair not only with its ordinary partner but with any of the other heterochromatic blocks. Each "illegal" pairing produces an exchange of partner, which greatly slows down the pairing of the euchromatic chromosome arms and is even likely to make it impossible in the most proximal regions. Thus crossing over only rarely takes place in these parts of the arms. The result is that gene loci are crowded around the centromere in the crossover maps. This state of affairs also affords an explanation of the phenomenon that interference, as a rule, does not work across the centromere, since it does not act over unpaired chromosome regions (cf. Oksala, Hereditas 38: 449-480, 1952).

The phenomenon of "interchromosomal effect on crossing over" implies especially the effect of inversion heterozygosity on the crossing-over frequency in heterologous chromosomes (see, e.g., the review by Schultz and Redfield, Cold Spring Harbor Symp. Quant. Biol. 16: 175-197, 1951). If one chromosome has a large enough inversion or two inversions, it pairs with its normal partner with an inversion loop(s). Such a configuration is not able to rise to the top of the bouquet but remains with its heterochromatic block at the base. In this case only four heterochromatic blocks are left at the top of the bouquet, and this gives them a better opportunity for "legal" pairing. The result is an easier and more complete pairing of the proximal euchromatin and thus an increased frequency of crossing over. If two chromosome pairs are heterozygous in regard to inversions, both remain at the base of the bouquet and the only homozygous pair alone rises to the top, having thus optimal chances for a complete pairing. Consequently, inversion heterozygosity in other chromosomes mechanically facilitates the pairing of the proximal parts of the arms of the remaining chromosomes causing in them a large relative increase in the crossing-over percentage. The other phenomena connected with this pronounced effect are explicable on the assumption that the interference pattern must be profoundly modified as a result of such a complete pairing.

Other findings related to "interchromosomal effects" are also explicable on the basis of the present theory, but they are not touched on in this limited exposition.

Okubo, S. A glucoside in D. melanogaster.

Metabolic products of tyrosine in D. melanogaster have been investigated by means of paper chromatography. About twenty pupae of various strains were extracted with 80% methanol, and the concentrated extracts were spotted on filter paper. The chromatograms were developed with n-butanol, acetic acid, and water (4:1:1) and then sprayed first with 2:6-dibromoquinone-chlorimide dissolved in butanol and subsequently with veronal buffer (pH 8.5). The blue spot was recognized at Rf 0.5. This spot is most evident in the claret (ca) strains, but indistinct in the ca^2 and other strains. A genetic analysis is in progress. As this compound is resolved into a diphenolic substance and a glucose after hydrolysis, it seems to be a glucoside similar to 3-hydroxy-4-0-(β -glycopyranosido)-benzoic acid isolated from the cockroach (P.C.J. Brunet & P.W. Kent, 1956).

Oster, Irwin I. Two unusual cases of white-variegation.

Thus far all known mutations resulting in the so-called variegated phenotype that have been tested for suppression of their mutant character by addition of heterochromatin (usually in the form of an extra Y chromosome) have proved to exhibit this reaction. These cases were originally called "eversporting displacements" by Muller because he had found that their mosaic expression (i.e., an interspersing of mutant and non-mutant tissues) was caused by a chromosomal disarrangement; later observations by him (1935) and by Schultz (1936), independently, showed that in each case the euchromatic locus in question had been placed in the neighborhood of heterochromatin.

However, in 1946 (DIS-20: 88) Muller found a sex-linked recessive mutation of spontaneous origin which causes alleles of white, such as apricot, to become mottled. This mutation, designated "mottler of the white series" (mw), is located slightly to the right of cut and is not associated with a structural change of the chromosome. By using a scheme for introducing extra Y chromosomes into the male we have found that extra heterochromatin is without effect in suppressing the action of mw.

The second case was found by Mrs. Astrid Cicak, working in our laboratory, amongst the F_1 offspring of Y^S.X InEN y.Y^L sc^8 y^+ (no free Y) irradiated males mated to y^2 su-w^a w^a bb.= (no free Y) females. This fly was a male with variegated eyes (i.e., mosaic for red and white facets). When bred to females with attached-X's it yielded males which all showed mottling of the eyes. However, only about half of them were non-yellow (y^+), whereas the rest had lost the sc^8 insertion (which carries y^+) and therefore were yellow (y). This variegated mutation, designated "white mottled of Cicak" (w^{mCi}), was found to have been caused by a change allelic to white. It was similar in appearance in both yellow and non-yellow lines. Although some double crossovers would be expected from breeding Y^S.X InEN w^{mCi} y.Y^L sc^8 y^+/y^2 v f car females, none were obtained in a total of 300 offspring; this indicates that w^{mCi} is associated with a large structural change of the chromosome. Introduction of an additional free Y into the stocks did not alter the expression of the variegation.

These observations indicate that the phenomenon of variegation in D. melanogaster is not necessarily associated with heterochromatic disarrangements that are suppressible by extra heterochromatin.

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Paik, Y. K. Identification of a few uncertain species of Drosophila reported in DIS-30.

In the last issue of DIS (p. 110) we reported a number of Drosophila species collected from May to October, 1956, of which some were recorded as uncertain species. We have identified these as follows: D. (Hirtodrosophila) sp. = D. (H) sexvittata; Amiota (Phortica) sp. = A. (P) variegata; Leucophenga sp. = L. (Trichiaspiphenga) argentosa; Mycodrosophila sp.--1 = Mycodrosophila poecilogastra.

Paik, Y. K. Seasonal changes in Drosophila populations in two adjacent areas in Korea.

The purpose of the present article is to report the results of a survey of seasonal changes in population size, and of the sex-ratio balance, of wild Drosophila populations. Samples were taken at two woodland areas around the foot of Mt. Mootung (1000 m in height), about five kilometers distant from the University. Collections were made, as a rule, at intervals of one week during the whole season from July, 1956, to June, 1957, by sweeping over large apple-baited trap-cans. At each of two areas four traps were placed in a row (10 m apart) at the fixed positions throughout the whole period. Baits were changed every week. All collections were done for three hours right before sunset in the late afternoon.

Our collections records show that a total of 12,918 flies were taken during the period in both areas. The area-1 collection consisted of 6082 flies, representing twenty-seven sympatric species, of which nineteen belonged to the genus Drosophila (including subgenera Drosophila, Sophophora, and Pholadoris) and eight to other genera of the family (Amiota, Mycodrosophila, Microdrosophila, and Leucophenga). The 6836 flies collected at area 2 represented twenty-five sympatric species, of which sixteen belonged to the genus Drosophila (including three subgenera, as in area 1) and eight to other genera (including the four found at area 1 plus Scaptomyza).

Changes at each of the two areas, showed two sharp seasonal maxima in size, one in the autumn (October-November) and the other in the spring (April). Total populations sank to an extremely low level, statistically zero, during the cold winter months (December-February), which can generally be considered a severe "population bottle-neck period" in our climate. Total populations also dwindled to a low level during the warm summer months (July-August). Results obtained here are in striking agreement with the pattern of seasonal changes in Drosophila populations of a temperate climate predicted by Professor Patterson (Univ. of Texas Pub. 4313: 203, 1943). The total population changes from month to month throughout the year were closely concordant with each other in the two populations at the two areas (correlation coefficient, $r = 0.969$ and $t = 12.402$).

Species-specific changes in the populations were also considered. Records of six species and two complexes of the genus Drosophila which were abundant or common throughout the year were selected for this purpose. Most of the selected species showed two yearly maxima, the rest one sharp maximum. Furthermore, monthly changes in relative frequencies were species specific. This is confirmed in some degree by computing the correlation coefficient (r) for relative frequency of a given species in the two areas. Some of the results are summarized in the first table. The data used for figuring the correlations were the numbers of flies of a given species collected in a given month divided by the total number of flies of the genus Drosophila collected in the same month.

	Relative Frequency area 1	Relative Frequency area 2	Seasonal peak	r	t
D. auraria	8%	11%	autumn and spring	0.964	11.463
D. transversa-complex	14	25	autumn and spring	0.928	7.876
D. nigromaculata	3	5	autumn and spring	0.763	3.439
D. cheda-lacertosa	2	3	autumn and spring	0.781	3.954
D. bizonata	30	30	winter and spring	0.998	49.921
D. coracina	30	16	spring	0.955	10.177
D. lutea	6	6	autumn	0.991	23.409
D. suzukii	5	4	autumn	0.874	5.687

D. bizonata represents an interesting case. This is the only species that was present throughout the whole year. Only one female was trapped at area 1 in February, when the mean temperature was below zero centigrade; none of any other species was trapped in this month. Nevertheless, this species was trapped at the two areas in considerable numbers during the rest of the "population bottle-neck period," during which cold weather near the freezing point continued. In addition to this species, out of ten rare species collected at either one or both areas, seven, including D. histrio, D. rubifrons, D. bifasciata, D. sternopleuralis (in Okada's MS), D. helvetica, D. sp. (quinaria section), and D. sp. (subgenus Drosophila), were collected sporadically only in the winter months. D. bizonata was the most abundant of these species adapted to winter environment.

The common and abundant species were again selected for a study of sex-ratio balance in the populations. Some of the results are summarized in the second table.

Species	Area	Females trapped	Males trapped	% female	% male	Chi square 1 d.f.	P
D. auraria	1	187	252	42	58	9.62	*
	2	239	431	36	64	55.02	*
D. lutea	1	163	228	42	58	10.81	*
	2	148	271	35	65	36.11	*
D. suzukii	1	60	250	19	81	116.45	*
	2	54	186	23	77	72.6	*
D. bizonata	1	1166	832	58	42	55.83	*
	2	1341	803	63	37	135.0	*
D. cheda-lacertosa	1	58	54	52	48	0.41	0.8-0.7
	2	110	74	60	40	7.04	*
D. nigromaculata	1	85	81	51	49	0.1	0.8-0.7
	2	183	166	52	48	0.83	0.5-0.3
D. transversa-complex	1	431	490	47	53	3.78	0.083-0.046
	2	761	906	46	54	12.61	*
D. coracina	1	776	840	48	52	2.54	0.157-0.083
	2	443	528	46	54	7.44	*
Totals	1	2926	3027	49	51	1.71	0.317-0.157
	2	3279	3365	49	51	1.11	0.317-0.157

* Probability much less than 0.01.

The deviation from the expected 50:50 sex ratio is striking in a number of species; but in the total number of flies collected it is not significant. Furthermore, female or male preponderance in each species is not random in the two populations at the two areas, but always consistent. Whenever a discrepancy between the sexes is apparent, it seems to b e due rather to a differential attraction to the bait than to a real preponderance of one sex; and the differential attraction to the bait seems to be species specific. A more critical study of this problem is being attempted.

Paik, Y. K., and Kim, K. W. Local key to species of Drosophilidae collected so far in South Korea.

Since 1955 we have carried on field collections in several parts of South Korea, and a considerable number of species have been taken. Some of the collection records were reported in DIS-30. As collection localities have been increased, we have attempted to survey the distribution patterns of these species, as summarized below. Roman numerals indicate (I) Mt. Moodung (located in Kwangju, Chunnam province),(II) Mt. Chiri (in Kurae, Chunnam province), (III) Mt. Hanra (in Chaeju, Quilpart Island), (IV) Mt. Sori (in Kwangnung, Kyongi province), and (V) Mt. Taepaik (in Hwangjee, Kangwon province).

Species	Localities				
	I	II	III	IV	V
Amiota alboguttata	-	+	-	-	-
A. variegata	+	+	-	+	+
Leucophenga argentosa	+	-	-	-	+
L. magnipalpis	+	-	-	-	-
L. concilia	+	-	-	-	-
L. maculata	+	-	-	-	-
L. quinquemaculipennia	-	+	-	-	-
L. ornatipennis	+	-	-	-	-
Mycodrosophila poecilogastra	+	-	-	-	-
Mycodrosophila sp.	+	-	-	-	-
Microdrosophila sp.-1	-	+	+	-	-
Microdrosophila congesta	-	+	-	-	+
Microdrosophila sp-2	-	-	+	-	-
Scaptomyza disticha	+	+	+	-	+
S. graminum	-	+	+	+	+
S. polygonia	-	-	-	-	+
Drosophila (H) alboralis	-	+	+	+	-
D. (H) sexvittata	+	-	+	+	+
D. (H) quadrivittata	-	+	-	-	-
D. (H) nokogiri	-	-	+	+	-
*D. sp. of Hirtodrosophila	-	+	-	+	-
D. sp. close to histrio	-	-	+	+	+
D. (Dorsilopha) buscki	+	-	-	-	-
D. (Pholadoris) coracina	+	+	-	+	+
D. (Pholadoris) puncticeps	+	-	-	-	-
D. (Pholadoris) rubifrons	+	-	-	-	-
D. (Sophophora) suzukii	+	+	+	+	+
D. (S) takahashii	+	-	-	-	-
D. (S) lutea	+	-	+	-	-
D. (S) melanogaster	+	-	+	+	-
D. (S) magnipectinata	-	-	+	-	-
D. (S) auraria	+	+	+	+	+

Species	Localities I	II	III	IV	V
D. (S) bifasciata	+	-	+	-	+
D. (S) sp. of obscura group	-	-	+	-	+
D. (S) helvetica	+	-	-	-	-
D. (Drosophila) transversa complex	+	+	+	+	+
D. (D) nigromaculata	+	-	-	-	+
D. (D) sp. close to kuntzei	-	-	-	-	+
D. (D) testacea	-	-	-	+	+
D. (D) bizonata	+	+	+	+	+
D. (D) makinoi	-	-	-	-	+
D. (D) immigrans	+	+	+	-	-
D. (D) sp. of immigrans group	-	-	+	-	-
D. (D) virilis	-	-	+	+	-
D. (D) sordidula	-	-	-	+	-
D. (D) lacertosa	+	-	+	+	+
D. (D) cheda	+	+	-	+	-
D. (D) repleta	-	-	+	-	-
D. (D) histrio	+	+	+	+	+
D. (D) sp. of quinaria section	+	+	+	+	-
**D. (D) sp.	+	-	-	-	-
D. sp. of Drosophila	+	-	-	-	-

*D. sp. of Hirtodrosophila is recorded in Okada's MS as D. histrioides.
**D.(D) sp. is recorded in Okada's MS as D. sternopleuralis.

Paré, J. P., and Bell, A. E. Lethal and sterility analysis of two populations of D. melanogaster under reciprocal selection for high fecundity.

In an experiment comparing various methods of selection for high fecundity, the population under individual and family selection was observed to plateau at generation 7 (Bell et al., 1955). A genetical analysis of this population by the marked-inversion technique revealed that neither lethal nor sterility genes contributed to this lack of response to selection (Brown and Bell, 1955). A statistical analysis of these data indicated that the plateau was caused by an exhaustion of additive genetic variation even though nonadditive genetic variation remained (Bell et al., 1957).

A second method of selection compared in the original experiment was reciprocal selection, which is designed to exploit both additive and non-additive genetic variation by selecting for crossing or combining ability between two segregating populations. Response to selection under this method, although more modest initially, continued over a period of 40 generations, and thus the performance under reciprocal selection eventually exceeded that of the closed population under individual and family selection. Response to reciprocal selection soon plateaued, however, even though apparent genetic variation remained in the segregating lines. Since the reproduction fitness of these lines as measured by fecundity and per cent emergence was observed to decline during the course of the experiment, the possibility of deleterious recessive genes could not be ignored. In order for these genes to be maintained at frequencies higher than their mutation rates, they would be expected to possess at least one of the following properties: (1) exist as balanced lethals; (2) contribute to heterozygote superiority for within-line reproductive fitness, $+/1 > +/+$; or (3) contribute to heterozygote superiority for fecundity in the cross-line progeny. This last point is significant in

view of the nature of reciprocal selection. Individuals selected to reproduce each segregating line are those whose cross-line daughters have high fecundity.

Using a marked-inversion technique similar to that described by Brown and Bell, 1955, eighty genomes were sampled from each of the two segregating lines under reciprocal selection, and carried through appropriate matings for producing "homozygous or isogenic" lines. Since some isogenic lines were derived from each segregating line, the suggestion of a balanced lethal mechanism is immediately ruled out. No sex-linked lethals were found. Tentatively, for chromosome 2, seven different (nonallelic) lethals have been identified in one line and four nonallelic lethals in the other line. Eight nonallelic third-chromosome lethals have been identified in the first line as compared with nine nonallelic third-chromosome lethals in the second line. None of the lethals found in either line were found to be allelic with any lethal from the other line. Matings are now being made to (1) measure the effect of these lethals when heterozygous on within-line reproductive fitness and (2) measure the effect of these lethals on fecundity when they are made heterozygous in cross progeny.

Plaine, Henry L., and Fradkin, Cheng-Mei. A sex-differential suppressor of erupt in the Swedish-b strain of D. melanogaster.

Practically all laboratory and wild strains of D. melanogaster tested carry the mutant gene "erupt" on the third chromosome and the suppressor of erupt on the second. The only difference in these strains is the strength of the alleles at the two loci; nevertheless, the suppressor alleles are universally effective against the erupt alleles with which they are by nature in combination in the different strains. When the two loci of the various strains are recombined, however, the degree of effectiveness of a suppressor allele varies, depending upon its strength and that of the erupt allele with which it is in new combination. The erupt mutant also becomes manifested when the action of its suppressor is blocked by certain treatments.

Regardless of the strain tested, or of the nature of the treatment used to induce the phenotype, no differences between males and females have previously been obtained in either frequency or expression of erupt eyes. It soon became apparent that there was a considerable sex difference among the affected flies of a Swedish-b strain in which erupt spontaneously appeared. After five generations of selection for the phenotype, the culture was distributed into and continued as six sub-strains, five of them being selected for and one against the phenotype. The expression is always more extreme in the females, and there is, moreover, a significant difference between the low frequencies of affected males and the high frequencies of affected females through more than 45 generations. It was originally thought that a mutation at the suppressor locus could not account for this sex difference (DIS-29). From analyses of chromosome substitution tests and outcross tests, it is now evident that the suppressor locus, or at least the chromosome on which it is located, is solely responsible for allowing the mutant to be expressed differently in the two sexes. When the X, 2nd, and 3rd chromosomes of the erupt and suppressor-erupt strains are respectively replaced with those from the Swedish-b strain, only the Swedish-b 2nd chromosome, with its suppressor of erupt, yields a difference between the sexes. In outcrosses, the sex difference is greater when the Swedish-b chromosome is derived from the female parent.

Plaine, Henry L., and Fradkin, Cheng-Mei. A high-mutating system in the Swedish-b strain of D. melanogaster.

Shortly after the appearance of erupt eyes in an otherwise wild-type Swedish-b strain (DIS-29 and this issue), a large number of spontaneous mutations were also obtained. To date, studies of the mutation rate per locus for eight loci on the third chromosome have given 2.2×10^{-5} as the male rate. For seven loci on the 2nd chromosome, an average rate of 2×10^{-4} has been obtained for the male. In another series, based on twelve loci, the male rate was 4.2×10^{-4}. For the female, the average second-chromosome rate was 7.4×10^{-5}, based on twelve loci but less than 9000 flies. In the male, where repeated backcrosses may be made, there appears to be an increase in mutations as the number of generations tested increases; that is, after the initial introduction of the mutator into the heterozygote, there appears to be an increase in mutation rate per generation, per generation--at least for some loci being tested. It is striking that both the sex-differential suppressor of erupt and the high-mutating system seem limited to the second chromosome!

(Supported by a grant from the National Science Foundation.)

Rasmuson, B. Genetic analysis of an isoxanthopterin-determining gene in D. melanogaster.

In an investigation concerning amino acid metabolism after treatment with organo-phosphorous insecticides, chromatographic separations of free amino acids in butanol : acetic acid : H_2O were made on some wild-type stocks of D. melanogaster. When examined in UV light, one of the fluorescent spots, the one due to presence of isoxanthopterin, was found to be almost absent in the Örebro stock. Head and abdomen were chromatographed separately for males and females of this stock and of a control stock containing normal amounts of isoxanthopterin. The difference could be attributed to the abdomen, and thus is probably due to the gonial pigmentation. It was much less pronounced in females than in males; the latter were therefore used in the following analysis. The amount of isoxanthopterin is highest in newly hatched males and decreases gradually with ageing. Animals of known age must therefore be used in quantitative estimations. The isoxanthropterin spot was eluted in NH_4OH and the intensity of the fluorescence measured with reference to a solution of kinin (0.25 µg/ml). For 8-day-old males the following values were obtained: Örebro stock--head 9.6, abdomen 6.8; control stock--head 7.9, abdomen 27.2. The gene responsible for the isoxanthopterin difference could be localized in the region between y and ec on the X chromosome. Among more than 200 crossovers from females heterozygous for the Örebro X chromosome and a chromosome containing y w^e ec, no crossing over was found between the isoxanthopterin gene and the white locus. It can thus be supposed that this gene is an allele of white, but it does not influence the pigmentation of the eyes. A decisive test of allelism must be made with females heterozygous for the isoxanthopterin gene and the different known pseudoalleles of the white locus. Such tests are under way, but the small amount of isoxanthopterin in the females makes the technique more involved.

Rasmuson, M. Unequal crossing over in the Bar region of D. melanogaster.

Unequal crossing over in the Bar region of the X chromosome gives rise to offspring with reverted + or BB eyes. The frequency of such crossover offspring was studied in crosses between f B od car/+ B + + females and f od car males,

with the intention of finding clusters of these phenotypes, which would indicate that crossing over had taken place in gonial cells. It was possible to raise the frequency significantly by means of high-temperature (31° C) treatment of the females for 48 hours. An increase from 0.133% to 0.206% was found in the offspring from eggs laid 5-10 days after the start of the heat treatment. It was accompanied by an increase of the crossover frequency in the region f to car. X-ray treatment (2500 and 4000 r) did not increase the frequency of unequal crossovers, nor the percentage of crossing over between f and car. The appearance of clusters of unequal crossovers was searched for by comparison with the Poisson distribution. The X-ray experiments, which were the most extensive, revealed a significant departure from the expected distribution. However, an analysis of the cases in which more than one unequal crossover appeared in a batch of offspring did not indicate any clusters that could have arisen from a single gonial crossing-over event.

Ronen, Amiram. Fluorescent substances in eyes of the Plum phenotype.

Chromatograms of heads of flies carrying the inversion Pm (dp, b, balancer) on a background of Berlin chromosomes were compared with chromatograms of wild-type Berlin. The solvent employed for unidimensional ascending chromatography was Propanol-1% ammonia solution (2:1) and the chromatograms were developed in an atmosphere saturated with collidine. In addition, separation was improved by the following procedure. After being run for 3 1/2 hours at 26° C, the chromatograms were dried, and a narrow strip, containing the start points and Fl 1, was severed horizontally from the upper part, containing Fl 3 and all subsequent spots. The upper strip was then further developed in the same dimension for another 2 1/2 hours. The fluorescent spots were cut out and eluted in 3 cc double distilled water each, and after 24 hours their fluorescence was measured in a Farrand Fluorometer. Filters employed for Fl 1 were 436 mμ as primary and 557 mμ as secondary, and those for all other spots were 365 mμ as primary and 436 mμ as secondary. The designation of the spots which is adopted here corresponds to that of Hadorn & Mitchell (1951) and Hadorn & Schwink (1956). A bluish fluorescent spot, which appeared between Fl 3 and Fl 4, and which was particularly distinct in chromatograms of the Plum phenotype, is provisionally referred to here as Fl 4 A. (Further tests are necessary before it can be decided whether Fl 4 A and Fl 4 B of the present note are identical with the two substances described by Forrest and Mitchell, 1955, under these names and identified by these authors as 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine and 2-amino-4-hydroxypteridine, respectively.)

Correction for possible differences in eye size between the two phenotypes could be dispensed with in the present case, since planimetric measurements of camera lucida drawings of the eyes indicated almost complete identity in size (mean of 30 eyes of Berlin males, 36.17±0.45; mean of 30 eyes of Plum males, 36.33±0.47).

Besides a marked reduction in the brown (density of start-points) and the red (Fl 1) pigments, the Plum phenotype is characterized by a relative increase in the concentrations of Fl 4 A and Fl 4 B and Fl 5 (see table). Fl 7 is also greatly increased in Plum chromatograms, but could not be measured with the available filters. A study of the Plum position effect in different genetic combinations should indicate whether normalization (increase) in the concentration of Fl 1 is correlated with normalization (decrease) in the amounts of Fl 4-5.

Following are the results of a comparison of 10 chromatograms each of four male heads of Berlin with 10 chromatograms each of four male heads of Pm.

Spot	Berlin flies	Pm flies
Fl 1	7.1±0.5	1.3±0.3
Fl 3	2.5±0.4	2.8±0.7
Fl 4 A	2.1±0.8	4.5±0.3
Fl 4 B	3.9±0.8	8.4±0.6
Fl 5	1.2±0.2	2.8±0.3

Ronen, Amiram. Variable success of crosses between D. melanogaster and D. simulans.

It is well known that the interspecific cross between D. melanogaster and D. simulans succeeds more readily when D. melanogaster is the female parent. Using virgin males and females of specific ages as proposed by Uphoff (1949), our mass matings in either direction have usually given results which were comparable to those of Sturtevant (1929) and of Uphoff. Thus one series of mel. x sim. matings yielded 63% of successes, whereas among the reciprocal crosses only 5% were fertile.

A strain of D. simulans collected in Tel-Aviv-Jaffa, Israel, by Mr. F. Gruber has recently given an unusual percentage of successes when utilized as female parent, and the results of the reciprocal cross are unexpectedly poor (see table). Since two different laboratory strains of D. melanogaster (Berlin and Cy L/Pm) were employed in each combination, we must conclude that the aberrant proportions of successes and failures were due to the genetic constitution of the simulans strain.

Type of cross	No. of "creamers"	No. fertile	% fertile	% females among hybrids
mel. x sim.				
Berlin x sim.	38	1	2.6%	100%
Cy L/Pm x sim.	33	1	3.0%	100%
sim. x mel.				
sim. x Berlin	69	36	52.2%	1.0%
sim. x Cy L/Pm	88	27	30.6%	0.5%

Sandler, L. Exchange in tandem compound ring X chromosomes.

A tandem compound ring X chromosome (without useful heterozygous markers) has been synthesized by Novitski (1954). This ring spontaneously converted, by crossing over, to a reversed compound ring, data from which have also been reported by Novitski. One of the major difficulties in interpreting these data is the lack of information on the relative viability of the compound ring-bearing female class. The present writer has recently made a study of newly synthesized reversed rings that do contain heterozygous markers, and by a comparison of the results of his study with that of Novitski, can make a strong argument indicating that there is no appreciable depression in viability of classes in the randem ring

experiments. This being so, it is interesting to re-examine, briefly, the results from those experiments.

Novitski's results (with all numbers corrected for meiotic loss) from a cross of females carrying a tandem ring and FR2 by YSX.YL, y B males are: regular ♂♂ = 3,012, exceptional ♂♂ = 302, matroclinous ♀♀ = 245, single ring-bearing ♂♂ = 913, single ring-bearing ♀♀ = 912.

From a tetrad analysis for the tandem ring (for details, see Novitski, 1954), it can be seen that one-eighth of all double exchanges result in equal bridges that yield exceptional males (Novitski, 1955). If there is no other source of such males, then the frequency of double exchanges in the tandem ring becomes 80 per cent (8 x 302/3,012); a surprisingly high value. Now, the tetrad analysis also shows that single rings produced from double-exchange tetrads come from dyads in which single rings separate either from single rings or from triple rings. In the latter case, it is probable that the single rings would be included in the functional egg nuclei with a frequency greater than 50 per cent (Novitski, 1951). It is, unfortunately, not possible to state exactly what this frequency is, but it has been shown (Novitski and Sandler, 1956) that when single rod chromatids separate from triple rod chromatids (in asymmetric dyads at anaphase II), the single rod is almost always recovered in preference to the triple rod. If this same situation obtains for the ring dyads, then it is the case that all the single rings recovered from the tandem ring are accounted for by double exchanges. It is true, however, that there is a deficiency of recovered tandem rings, the reason for which is not obvious at present.

Although it is very possible that there are other sources of exceptional males in tandem ring experiments, and it is also possible that the degree of nonrandom disjunction is less than that postulated here (either possibility would probably be indicative of some frequency of single exchanges), it is also conceivable that these estimates are reasonable, in which case the reduction (or absence) of single exchanges previously noted for reversed acrocentric and reversed ring compounds (Sandler, 1954 and in press) might extend also to tandem ring compounds.

Schalet, Abraham P. A spontaneous inter-chromatid exchange involving the Notch region.

Among the offspring of a cross of males (P_1), $1J1^{+}.Y/\overline{1J1}$ sc^{J1} In49 B^{M1}, by females (P_1), $\overline{1J1}$ sc^{J1} In49 v B^{M1}/"Maxy-$\overline{1}$" (see Muller and Schalet, this issue) a single mosaic female was found, having one wing showing a typical Notch phenotype with respect to notching of the tip and thickened veins with deltas. The other wing was closely examined and appeared to be wild type; furthermore, no noticeable irregularity of thoracic hairs or of either eye was detected. The female had already mated with a brother (P_2), $\overline{1J1}+Y/\overline{1J1}$ sc^{J1} In49 v B^{M1}, but was in addition crossed with other males (P_2) $\overline{sc^{8}}.Y/\overline{y}$ sc^{S1} B InS.

From these matings at least two derivatives of the original X chromosome from the P_1 male were recovered. Stock 1: All F_2 males that survived to the imaginal stage showed a variable irregular thickening or extra veins along one or more longitudinal veins and sometimes at the distal ends of the veins. Irregular thickenings or deltas frequently were present at crossveins. Some F_2 females showed the same characteristics as the males, but to a lesser degree and grading to wild type. Stock 2: Other F_2 females showed a typical Notch phenotype with respect to wing characters and thoracic hairs at least.

Males from stock 1 were crossed to females from a spontaneously arisen Notch stock of independent origin in which all the females regularly show a Notch phenotype. However, all female offspring of this cross were wild type even though they carried a Notch chromosome. Females from stock 2 were crossed with split (spl) males. The pseudodominant expression of split in the female offspring receiving the Notch-type chromosome confirmed the genotypic designation of Notch. Unfortunately, stock 2 was accidentally lost soon after this confirmation was made.

These results indicate that the derivative of the original paternal X chromosome represented by the chromosome of stock 1 carries a duplication for the Notch region. This interpretation is supported by the similarity, in its phenotypic expression and suppressor action, to the published reports of Dp(1;1)Confluens found by Gottschewski and analyzed by Schultz (see Bridges and Brehme). In the present case, designated Confluens^2, a crude localization has placed the duplication at 4.5±1.1 units from yellow. Furthermore, in addition to the duplicated chromosome being present in a portion of the gonads of the F_1 female, another portion of the gonads and also a part of the soma must have carried a chromosome deficient for at least part of the region represented by the duplication.

As in the original Bar case, the interchange must have arisen in a mature spermatozoon or one of the initial cleavage stages as a consequence of two nearby breaks in a chromosome before or after its formation of chromatids. If the breaks occurred before chromatid formation, the small piece between the breaks of one of the resulting chromatids could have been inserted into the sister chromatid, along with the piece proper to it, to produce a duplication in tandem or in reverse order. This would produce a complementary deficiency in the other chromatid. In the event that two very close breaks were produced in each chromatid of an already duplicated chromosome, an asymmetric rejoining of broken ends would produce one tandemly duplicated chromatid and one deficient chromatid.

Thus far no attempt has been made to obtain females homozygous for the duplicated chromosome, to test for crossing over with the recessives of the Notch region or to obtain reversions to wild type or changes to more extreme type by unequal crossing over. Confluens^2 is represented (as stock f28) in the current Bloomington stock list and will be available for anyone desiring to analyze the duplicated region cytologically.

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Seto, F. Delay in pupation of pupal lethals.

It was observed in the study of some balanced lethal strains that lethal pupae were found consistently in the lower part of the culture vial, near or in the food, whereas the Cy/l pupae were found higher up the wall of the container. Furthermore it had been noticed in previous observations of crowded cultures that the earlier-emerging larvae pupated at higher levels than those that emerged later, many of the latter pupating in the food itself. This difference in pupation levels between the lehtal homozygotes and heterozygotes was perhaps indicative of a difference in pupation time. The validity of this supposition was tested in the following simple manner. Eggs from the balanced stocks were collected within a 12-hour period and incubated in vial cultures at 25° C. Upon emergence of the larvae from the food, they were separated into two

groups: vial A, containing the larvae which pupated within the first 24 hours; and vial B, containing the remaining larvae. The separation was accomplished by either removing the larvae individually or scooping up the food with the remaining larvae and transferring them to a second vial (B). Since practically all the larvae of strains N1A, N55, and N25 emerged from the food during the first 24 hours, the procedure had to be modified by shortening the separation time from 24 hours to 12 hours.

In all cases vial B contained a higher percentage of lethals than vial A. This difference clearly indicates that the lethal larvae emerged to pupate later than their heterozygous culture mates. Moreover, it appears that the lethals with the earlier-acting effects show greater delay in pupation than those with effects appearing later in development. The study indicates that part of the total pattern of damage of pupal lethal factors extends to a varying degree into the "larval stage" and influences the time of emergence.

Seto, F. Effects of crowding on the time of action of some pupal lethals. In a previous study in which the times of action of recessive lethals were determined, vial cultures of various population sizes were used to obtain the data. It was noticed at that time that crowding tended to reduce the number of lethal homozygotes appearing at the expected stage of development. To test whether this crowding effect was important in altering the time of action and if it was equally effective for all the pupal lethals studied, the following plan was carried out. Vials containing the same amount of food but varying in the number of eggs were started. At intervals, egg-hatch counts, pupa counts, and adult counts were made. Special attention was given to the time of expression of the lethals within the pupation period. Four population sizes in replicates were studied: 25 in 30 replicates, 50 in 20 replicates, 100 in 10 replicates, and 200 in 10 replicates. Population size of 400 was started, but the excessive mortality and overcrowding made scoring difficult. In a few strains the proportion of lethals was the same in all four population sizes; in others, with an increase in number there was either a reduction in the proportion of lethals or the lethals did not develop as far as expected. The results of the study of thirteen pupal lethals are summarized in the table. (L-Pr = larval-prepupal; EP = early pupal; LP = late pupal; I = imaginal.)

Lethals	Time of action	No effect	Reduction in no. of lethals	Earlier expressions of the lethals
N42A	L-Pr	*	-	-
N61	L-Pr	*	-	-
X3	L-Pr	*	-	-
N51	L-Pr	-	*	-
N50	EP	-	*	-
N32	EP, (LP)	-	-	Fewer LP's
N4	EP, (LP)	-	-	Fewer LP's
Co7	EP, (LP)	-	-	Very few LP's
Co3A	EP, LP	-	-	Fewer LP's
N1A	LP	-	-	Fewer LP's, many EP's
N55	LP, (I)	-	-	Many EP's
N13	LP, I	-	*	-
N45	LP, I	*	-*	-

In the lethals which normally show their effects before the eversion of the cephalic complex (larval-prepupal lethals) or at the time the adults emerge, there appears to be no change due to crowding. In the others, with an increase in population size (especially at 200), there is either a reduction in the number of lethals or an earlier manifestation of lethality. In the lethals which display their effects during early and late pupation, crowding increases the early class at the expense of the late class. The apparently negative results for the three L-Pr (N42A, N61, and X3) and the one LP (N45) lethals may simply indicate inability to score the particular stage of action. It is not possible to partition the prepupal stage as can be done with the pupal stage, which is of long duration and has early, middle, and late phases that can be easily distinguished by visible changes in the developing fly. Although no details are presented here, crowding has only a minor effect. Although the number of lethals and/or the time of expression may be modified, each lethal still manifests itself within a definite ontogenetic stage.

Work is now under way to determine the effects of pupal lethals in combination. The preliminary results indicate that there is no complementary effect between the lethals taken two at a time which extends the development of the pupae. As would be expected, the time of action is determined by the earlier-acting lethal, and often the combination results in an earlier cessation of development than is manifested by either lethal singly.

Shiomi, T. Lethal effect of In(1)-T(1,4) in D. virilis. This strain was obtained by Oshima through repeated crosses between D. virilis and D. texana. A distal translocation is present between the X and fourth chromosomes, and an inversion ranging from the break point of the translocation to the proximal end of the euchromatic region is recognized on the X chromosome. The hemizygous male and the homozygous female as regards the aberrant X are lethal. The lethal effect appears at two embryonic stages; first, before blastoderm formation; and second, about the stage of tracheal formation. Approximately a quarter of the eggs do not form the blastoderm, and most of the nuclei formed inside may not be able to migrate towards the periphery of the egg. At about the stage when the tracheal system becomes almost complete, retardation of development is observed in 20% of the eggs, which fail to hatch. The few larvae which do hatch from such eggs soon die.

Sondhi, K. C. Identification of the fourth linkage group in D. subobscura with element E. Flies homozygous for the mutants poppy and pointed, from the fourth linkage group, were crossed to the B and K inbred lines, both structurally homozygous for all the chromosomes. From the F_1 two sets of backcrosses were made and fifteen cultures of each were counted. The following results were obtained:

	n.c.o.	c.o.	Total	Recombination
B	2150	758	2908	26.06%
K	3160	1060	4224	25.09%

Maynard Smith, Clarke, and Hollingsworth (1955) described the chromosome orders for the B and K inbred lines. According to them, only the E chromosome, out of the four long autosomes, has the same order (alpha) for both the B and K inbred lines. The fact that pp pt yield the same recombination values with the B and K inbred lines is suggestive of their identification

with element E. As a check, the pp pt flies were also crossed to the NFS inbred line, which has a different order for element E. F_1 flies were backcrossed to pp pt, and fifteen cultures were counted. Out of 2820 flies, only 4 crossovers were obtained. This confirms the previous findings.

Spiess, E. B. Effect of Tegosept M on rate of development in D. persimilis.

During experiments on survival and rate of development of pre-adult stages in D. persimilis at 16° C, it was accidentally discovered that the amount of mold preventive (Tegosept M) being used was only 10% of the amount specified in the food formula (cream of wheat-molasses: Spassky, DIS-17, 1943). As soon as the correction was made and the amount of Tegosept was multiplied by ten, it was noted that a number of strains were slowed down in development. To be certain that the retardation was brought about by the Tegosept and not by some temperature or other environmental fluctuation, the following test was made at cold temperature (12° C). Two "normal" strains of homokaryotype WT persimilis were mated (about one dozen pairs per half-pint bottle) and food was provided on plastic spoons. Eggs were collected every day and planted in lots of ten, five lots per vial, on the surface of slanted food medium in glass vials (9.5 x 2 cm). Tegosept was added to the cream of wheat-molasses food in the following proportions to the Spassky formula: 0x, 0.1x, 1x, and 2x. (The Spassky medium calls for Moldex at about 0.06% of the liquid constituents by weight.) Ten vials containing 50 eggs each were made up for each proportion, and all were run simultaneously. The following data on survival and rates of development were collected. (N = average no. survivors; Days = average no. days from egg to adult.)

0 x		0.1 x		1 x		2 x	
N	Days	N	Days	N	Days	N	Days
15.4±2.2	36.7±.38	21.2±2.9	36.4±.55	23.3±3.4	38.6±.64	24.0±2.6	61.3±1.87

None of the differences in survivors is significant. (The largest difference, 5.8 between 0x and 0.1x, gives a "t" value of 1.606, d.f. 18, p = 0.12.) Cultures with 1x Tegosept took longer to develop than cultures with no mold preventive or only a trace (0.1x). The difference of 2.2 days is highly significant, with a "t" value of 2.51 (d.f. 18, p = 0.02.) Cultures with 2x Tegosept took about 1.6x the days to develop that cultures with 1x Tegosept took, or about 1.7x as long as cultures lacking Tegosept. Tegosept is not toxic, apparently, but must be used with care in developmental rate studies. The trend of increase of average survivors with increasing Tegosept may be real in that the mold preventive suppresses growth of microorganism contaminants which might have antibiotic activity on the larvae.

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Strangio, V. A. Genic interaction in D. melanogaster.

The researches of Stern (1943) and House (1953) on the progressive obliteration of the distal tip of the fourth longitudinal vein in D. melanogaster have established the existence of an unidentified biochemical substance, P, responsible for the elaboration of pupal lacunae by a patterned inhibition of certain cells lying opposite one another in the dorsal and ventral laminae. Recent work in this laboratory has

centered on the behavior of P above the postulated "100%" level of the normal phenotype (House, 1954). It is suggested that excess P invades the erstwhile intervein material, producing extra venation and/or a weakening of interlaminal adhesion, with consequent blister formation during wing expansion. Investigation has shown that, in the heterozygous condition, each of three wing mutants at the extreme right end of the second chromosome (plexus, blistered, and balloon) induces a significant exaggeration of the third-chromosome dominant, Delta (Dl^3), based on the presence or absence of large blisters in the adult wing. From a quantitative aspect, this method of estimating P-level for various markers is far from satisfactory. Further work is planned along lines likely to provide a more rigorous statistical basis by means of cancellation in plus/minus-P combinations with one group marker as a standard level (cf. the Delta-Hairless interaction).

Taira, T., Nawa, S., and Oshima, C. Pterin dehydrogenase in eye-color mutants of D. melanogaster.

The activity of pterin dehydrogenase in Oregon-R and in the eye-color mutants v, se Hn^{r3}, ry, bw, w, and w^a, was investigated by means of both paper chromatography and comparative fluorometrical measurements. It was found that pterin dehydrogenase had the highest activity at the pupal stage, and Oregon-R had the same enzymatic activity as v, se, bw, w, and w^a. However, Hn^{r3} had less coenzyme than the other strains. Furthermore, ry showed hardly any activity of the enzyme. This corresponds to the fact that isoxanthopterin was not detected in the body of ry, although AHP (2-amino-4-hydroxypteridine) was found in its body.

Takada, H., and Okada, T. Occurrence of a new species of the virilis group in Hokkaido.

Twelve specimens, each of which is closely related to D. littoralis were collected in traps at eight districts in Hokkaido. They will be reported as a new species, with the name D. ezoana, belonging to the virilis group. The phallic organs and egg-guide of this species are characterized as follows.

Phallic organs: Aedeagus pale brown, large, fusiform; darker at margin, and apically with divergent pale processes. Basal apodeme of aedeagus about half of total length. Anterior paramere small, pale brownish grey, fused to novasternum. Novasternal or hypandrial plates dark brown, pubescent, and each with a long stout submedian spine. Ventral phragma pale brown, darker at margin, nearly as long as broad, proximally narrowing with convergent margins, laterally almost straight, and rounded at the anterior tip.

Egg-guide: Lobe broadest subapically, rather rounded apically, tapering basally, and with about 18 marginal and 2 distal pointed teeth. Subterminal hair located near the tip of the lobe. Basal isthmus narrow and very short.

Tantawy, A. O. Crosses between parents of different body size, selected from random inbred lines of D. melanogaster.

An inbreeding experiment (random matings) was carried out in which two different systems of matings were used, namely, brother-sister and double-first-cousins. In each system five parallel random-mated lines were maintained. At 25, 50, and 75% coefficients of inbreeding, adults were measured and selected for long,

medium, and short wing length. Such selected parents (large, medium, small) were crossed within each system of mating, that is, large x large, medium x medium, and small x small. The ten possible crosses were carried out, and heterosis was measured as ratio of average F_1 to average parent size. The heterotic effect on wing length in such crosses is shown in the table.

	Brother-sister			Double-first-cousins		
F_x	25%	50%	75%	25%	50%	75%
Large x L	0.26	1.64	4.57	-1.14	0.68	0.97
Medium x M	0.83	1.42	3.98	-1.74	0.66	1.42
Small x S	0.92	1.44	3.93	-2.15	0.71	1.04

It is clearly shown that when the selected parents were crossed within each system, heterosis occurred in both cases. Heterosis of wing length increased gradually with increased homozygosity, and matings between sibs displayed greater heterosis than those between double cousins.

Selection of parents in crosses gives more heterosis than inbred lines crossed at random (DIS-29). The same results have been obtained with regard to percentage of emergence; those results are in preparation and will be published soon.

Tantawy, A. O. Limits of selection with sib matings in D. melanogaster.

Long selection experiments have been carried out to investigate limits of selection with brother-sister matings. Two identical experiments have been maintained with four different selected lines, two of which were selected for long wings and the other two for short wings. The first experiment was carried out for twenty generations and the second for twenty-five generations. Before selection was started, heritability estimates were investigated by six progeny tests. The weighted means indicate that 43% and 45% of the total variances for wing and thorax length, respectively, are apparently due to additive genetic variance.

The results indicate clearly that selection is effective in both directions; selection for small size showed greater response than selection for large size. In all lines, selection limits were achieved at the fourth and fifth generations, respectively, in the case of long and short wings, after which there was a tendency for all lines to remain constant in size to the end of the experiments.

Phenotypic variance declined in the earlier generations of inbreeding and selection, after which it remained almost constant. When selection was relaxed, at the lower levels of inbreeding, all the characters studied returned back completely to the control level, whereas at higher levels relaxed selection had no effects.

Heritability of wing length was estimated at different levels of homozygosity, and the results are presented in the first table. It can be seen that heritability estimates declined in all the selected lines after the fifth generation, that is, 67.2% of inbreeding. In both experiments short-wing selected lines showed higher estimates than long-wing selected lines.

Generation	F_x	Experiment I Long	Experiment I Short	Experiment II Long	Experiment II Short
1	25.0	38.5 ± 5	42.3 ± 4	35.2 ± 4	40.5 ± 3
5	67.2	19.4 ± 6	23.2 ± 7	17.2 ± 4	25.3 ± 5
10	88.6	6.2 ± 4	10.3 ± 6	5.3 ± 2	10.2 ± 3
15	96.1	4.3 ± 4	7.6 ± 4	4.2 ± 3	8.3 ± 2
20	98.1	5.2 ± 2	5.6 ± 3	6.2 ± 4	5.2 ± 4
25	99.5	----	----	3.1 ± 2	4.3 ± 2

When selection was relaxed for five successive generations at the previous levels of homozygosity, the values for heritability estimates shown in the following table were obtained.

F_x	Experiment I Long	Experiment I Short	Experiment II Long	Experiment II Short
25.0	40.2 ± 5	44.2 ± 3	45.2 ± 3	50.2 ± 4
78.5	10.3 ± 4	16.3 ± 4	12.3 ± 4	10.3 ± 5
88.6	6.2 ± 3	8.3 ± 4	4.4 ± 4	8.2 ± 2
98.6	4.2 ± 2	3.0 ± 2	5.2 ± 3	5.5 ± 3

The results indicate that relaxed selection caused an increase in the heritability estimates at the lower level of inbreeding, that is, 25%, whereas at higher levels relaxed selection had practically no effect on the genetic variance of the selected lines.

Tantawy, A. O., and Mallah, G. S. Genetic resistance to heat in natural populations of D. melanogaster and D. simulans.

Natural populations of D. melanogaster and D. simulans were sampled from different geographical regions--one each from Lebanon and Uganda and three from Egypt. The Egyptian populations were from: the University of Alexandria Farm; Wadi El Natroon, a desert isolated area halfway between Alexandria and Cairo; and Luxor, about 550 km south of Cairo. These five localities are designated as LB, UG, UF, WD, and LX, respectively.

D. melanogaster and D. simulans were found in all these localities except LX, where only melanogaster was found. However, a simulans population from Beni-Swef (BS), about 112 km south of Cairo, was used.

All these ten populations were exposed to different temperatures--18°, 22°, 25°, 28°, 30°, and 31° C. The experiment is still going on, and lower and higher temperatures will be used. The characters being studied are: body size, that is, wing and thorax length; percentage of hatchability; and percentage of sterility. The results for wing length are presented in the table, as averages for both sexes, since males and females show almost the same reactions. The unit of measurement is 1/100 mm.

(see table on following page)

D. melanogaster

Locality	18° C	22°	25°	28°	30°	31°
WD	223.45	202.48	189.34	179.00	165.88	152.66
UF	223.00	201.57	189.39	175.63	168.16	158.90
IX	225.37	204.43	188.27	176.18	167.72	158.04
LB	235.35	211.71	197.25	185.13	173.48	164.40
UG	224.22	206.53	195.15	187.80	178.84	171.06

D. simulans

Locality	18°	22°	25°	28°
WD	204.23	197.30	174.30	147.99
UF	199.12	204.01	173.57	158.38
BS	201.04	203.21	174.77	158.99
LB	202.82	205.56	176.20	159.01
UG	214.16	200.73	186.17	166.55

The results demonstrate clearly that there are significant differences in wing length within all populations at different temperatures, that is, wing length is much greater at lower temperatures. D. melanogaster from LB has the longest wings at lower temperatures, and that from UG has the longest at higher temperatures. It can also be seen that D. melanogaster is larger than D. simulans. D.m. showed higher percentages of emergence than D.s.; this character appears to be greatly affected in all populations of D.m. and D.s. by higher temperatures. The best fertility was achieved at 25° in D.m. and 18° in D.s. Percentage of sterility increased with increase in temperature. In all the populations of D.m. studied, complete sterility was reached at between 30° and 31°. In four of the populations of D.s., it was reached at between 28° and 29°, but D.s. from the locality of UF is still fertile at 29° C.

These experiments are still under way, and the full results will be published very soon.

Thoday, J. M. Effects of stabilizing and disruptive selection.

Stabilizing selection is here defined as selection which favors the same (mean) phenotype in every generation. Disruptive selection is defined as selection which picks out both extreme phenotypes in every generation. A population of D. melanogaster has been exposed to disruptive selection by mating opposite extremes for 35 generations (disruptive selection with negative assortative mating). Another has been exposed to disruptive selection for 23 generations by mating like extremes together and selecting both extremes from the offspring (disruptive selection with positive assortative mating). A third has been exposed to 23 generations of stabilizing selection. All are derived from the same wild stock. The character selected for measurement was number of sternopleural chaetae. Four single-pair cultures are used each generation. Heritability has been tested in these populations by taking out high and low directional selection lines and recording the divergence of their mean chaeta numbers. Divergence is greatest in the disruptive selection lines, least in the stabilized lines, and (at least in the first

generation) intermediate in the foundation wild stock. It therefore seems that disruptive selection may increase and stabilizing selection decrease the effective variety of chromosomes in a Mendelian population. Disruptive selection may therefore be capable of producing polymorphism, as Mather has suggested.

Toyofuku, Y. Further observations on chromosomal polymorphism in natural populations of Drosophila in Hokkaido.

In addition to those reported in DIS-30, thirteen different kinds of variations have been found by investigation of salivary-gland chromosomes. Among them were nine types of variation occurring in five strains of D. nigromaculata. The remainder were observed in D. auraria, D. coracina, D. funebris, and D. histrioides.

Tsukamoto, M. Cross resistance to insecticides in D. melanogaster.

In order to ascertain the relation between the DDT-resistance gene on the second chromosome and cross resistance to various synthetic insecticides, several series of selections with DDT, BHC, and Dipterex were carried out in the laboratory. A wild-type population established from a mixture of various strains other than the most resistant Hikone-R strain was divided into several groups, and selections were started in 1955-1956. After intensive selection with each insecticide, these stocks developed levels of resistance as high as the multiple-resistant Hikone-R strain, not only to the selected insecticide but also to DDT.

Genetic analyses suggest that DDT resistance and BHC resistance in the BHC-selected strain depend upon a second-chromosome factor, and that Dipterex resistance and DDT resistance in the Dipterex-selected strain are also linked with the second chromosome. The effect of other chromosomes, if any, is not so significant to resistance. Although an analysis for location on the chromosome has not been made, it is thought likely that these resistance factors have the same locus or closely linked loci, because of the similarity in development of resistance to the insecticides.

Ulrich, Hans. The influence of oxygen on the lethal action of X-rays on nucleus and cytoplasm of uncleaved Drosophila eggs.

Anterior and posterior halves of Drosophila eggs, 10-20 minutes old at the beginning of exposure, were X-rayed separately with various doses (50 kv, 10 ma, filter 1 mm Cellon, time of exposure constantly 3 min., target distance varied) in atmospheres of air or nitrogen. At this age the anterior half of the egg contains the nucleus (the two pronuclei) at stages varying between meiosis and first cleavage, whereas the posterior half does not contain any nucleus. The frequencies of nonhatching eggs were registered and corrected according to the rate of nonhatching eggs in non-X-rayed controls. The results of one experiment are tabulated below.

As reported previously (DIS-29, 170-171), the dose-effect curves obtained by X-raying the anterior or the posterior halves in air differ quantitatively and qualitatively. The new results show that the lethal action of X-rays is reduced by nitrogen in the case of the anterior halves as well as the posterior halves. The specific shapes of the dose-effect curves of the two halves obtained by X-raying in normal air are not modified by nitrogen.

Plotted semilogarithmically, both curves (air and N_2) of the anterior halves are nearly straight lines. Both curves (air and N_2) of the posterior halves, on the contrary, rise concavely in semilogarithmic plot.

Anterior halves X-rayed			Posterior halves X-rayed		
Dose r	In air %	In nitrogen %	Dose kr	In air %	In nitrogen %
100	11.0	9.8	20	18.0	19.6
200	31.7	9.8	40	37.3	12.7
400	43.7	16.7	60	39.8	19.9
600	70.2	35.2	80	46.8	32.1
800	70.8	39.6	100	47.4	27.8
1000	80.0	55.9	120	57.9	37.7
1200	79.8	60.8	140	74.3	47.4
1400	77.0	65.9	160	85.7	60.4
1600	85.1	68.4	180	95.8	83.5
2000	90.3	74.0	200	95.3	78.7
2400	87.4	78.0	240	97.7	93.0
2800	85.5	85.7	280	98.5	99.9
LD^{50} about	500 r	950 r	LD^{50} about	95,000 r	140,000 r

Walen, Kirsten H. Unexpected mosaicism for y with four doses of y^+.

Flies of the constitution sc^7 w^a/sc^7 w^a with a centric ring fragment, y^+ ac^+ sc^+, and T(1;4) y^+ ac^+/ci ey^D had four representatives of the y^+ locus. Abdomen mounts showed about 211% mosaicism for yellow bristles, that is, on the average 2 yellow bristles per abdomen. This percentage is somewhat higher than that usually obtained with such mosaic-producing processes as somatic crossing over. Mosaicism for y was not found with the sc^7 chromosomes alone or with the addition of either small duplication; it seems unlikely that the y spots can be attributed to position effect of the variegated type or to the somatic loss of either or both of the small duplications. The possibility of interaction of plus alleles to produce a recessive phenotype is being further investigated. Mosaicism for hairless spots, characteristic of the "shaved" appearance of sc^7, was found in 90 per cent of abdomen, and can readily be explained as loss of the centric ring fragment.

Williamson, D. L. Incidence of CO_2 sensitivity in several Drosophila species.

Carbon dioxide sensitivity (death in CO_2 at 13° C) has been found in wild-caught D. melanogaster, affinis, and athabasca, and also in laboratory strains of tolteca. The inheritance of this sensitivity in affinis, athabasca, and tolteca has not yet been determined but is being investigated. (The author is indebted to Dr. Nadine Plus, Laboratoire de Génétique Formelle, Gif-sur-Yvette, France, who, while at the University of Nebraska in 1956-57, instigated the author's interest in this research and kindly taught him the procedure and technique.)

Wild-caught melanogaster from Lincoln during August exhibited 1.97% sensitivity (84/4248) and during September .87% (18/2053). CO_2 sensitivity

was found in smaller samples (October, 1957) from Beatrice (14/263 = 5.3%) and Blair (4/38 = 10.5%), Nebraska.

Wild-caught affinis from Chadron State Park, Nebraska (June and July, 1957) exhibited 25% sensitivity (41/163) and small samples from Lincoln (June, August, and September, 1957) 24% sensitivity (19/79).

Several samples of wild-caught athabasca from Alaska (July and August, 1957) have shown CO_2 sensitivity: 2 out of 47 (4%) from College, 38 out of 441 (8.6%) from the Matanuska Valley, and 52 out of 591 (8.8%) from Big Lake. (These were kindly furnished by Dr. D. D. Miller.)

Two laboratory strains of tolteca, Santa Maria de Ostuma (Nicaragua) and Chapulhuacan (Hidalgo, Mexico), were found to contain flies sensitive to CO_2: Santa Maria de Ostuma, 24 out of 469 (5%); and Chapulhuacan, 14 out of 167 (8%). (These strains were kindly furnished by Drs. William Heed and Marshall Wheeler.)

Wolfson, M., Stalker, H. D., and Carson, H. L. A serious parasite of laboratory Drosophila.

In this laboratory recent investigations with D. parthenogenetica have resulted in the discovery of a parasitic protozoan, possibly a microsporidian, which may result in a type of parasitic castration of males and may also drastically affect the longevity of flies of both sexes, depending upon the degree of infection. Infection may be so serious as to prevent hatching of pupae. Infected individuals can be recognized, upon dissection in saline, by the presence of spores in the tissues and body fluid. The spores are easily identified by their strikingly consistent size and shape and by an extremely thick and rigid capsule. They are ovoid in shape, 4-5 μ in length, and may occur singly in the body cavity or associated with tumor-like structures.

Spores were first noticed in the adult testis. In less severe cases, the infection may appear as growths on the surface of the testis. In more severe cases, the infection fills the lumen of the testis and is generally localized in the lower two-thirds of the testis just to the point of union with the seminal vesicle. Infection may be bilateral or unilateral and may differ in degree in the two sides. Unilateral and bilateral rudimentary adult testes were also observed and appeared to be the result of an early infection of the larval testis. Spores were also found to be associated with the adult fat bodies. Larvae from infected cultures contain spores in the lumen and wall of the intestine, and spores have also been found in the lateral oviducts of adult females; this may indicate infection per os and/or direct infection of ova.

This infection has also been noted in a strain of D. melanogaster (subgenus Sophophora), resulting in a marked mortality. Young larvae of D. paramelanica may be infected if grown on spore-containing food. Efforts are being made to determine the manner of infection and methods of control and extinction. It is hoped that this brief note will serve to alert other Drosophila workers.

Research Note - Received Late

Fritz-Niggli, Hedi. Qualitative and quantitative differences in the induction of dominant lethals in Drosophila by 31-MeV X-rays, 30-MeV electrons, and 180-keV X-rays.

In connection with other tests, we were able to prove the effectiveness of 30-MeV electrons and 31-MeV X-rays from a Betatron, as compared with ordinary X-rays, in inducing dominant lethals in Drosophila. The test consists in determining the percentage of unhatched embryos in different periods after irradiation of the male parents. We mated 15 irradiated males (0-4 hours old), immediately after treatment, with 20 virgin females (3 days old) of an inbred stock (Sevelen). On the 4th, 7th, and 10th day the females were removed and new virgin females (3 days old) were mated with the irradiated males. Every day (28 hours after deposition of the last egg) we counted dead and living embryos hatched.

By this method we obtained the most exact radiobiological test with which we have ever worked. The experiments, repeated in six series with intervals of one month between, gave results with a variability for certain points of only ±2% or less. The accuracy of this radiobiological experiment is usually found only in physical measurements. We irradiated with 30-MeV electrons (91 rad/min.) in air, with 31-MeV X-rays in a plexiglass container at a depth of 40.5 mm (95-108 rad/min.), and with 180-keV X-rays in a plexiglass container at a depth of 12.5 mm (78-102 rad/min.) (30- and 31-MeV X-rays from the Brown, Boveri Betatron). The dose measurements were done with a Victoreen condenser r-meter for 31-MeV X-rays and 180 keV, and simultaneously for electrons with an integrator (thin-walled ionization-chamber) calibrated with the Victoreen condenser r-meter.

In table 1 we can see a strong dependence of hatchability on the stage at which sperm are irradiated (1000 r ionization dose). We can see a low efficiency for all three types of radiation in the first brood (irradiated mature sperms), then an increase of sensitivity in the second brood from the 4th to the 7th day. In the first brood a sharp increase was observed at the 3rd day, but only after irradiation with rays of high energy. After the 4th to the 7th day the efficiencies of 31-MeV X-rays and 30-MeV electrons remained the same, in contrast to the higher efficiency of 180 keV.

Between the 7th and 10th days we found a different action for every type of radiation. The most effective were the 180-keV rays, then the 30-MeV electrons, and last the 31-MeV X-rays. The peak of sensitivity for 30-MeV electrons was later than the peak for the 31-MeV X-rays. The efficiencies of these two types of radiation must be qualitatively different.

Table 2 shows the relation of efficiency of 30-MeV electrons or 31-MeV X-rays to 180 keV with regard to RBE (relative biological effectiveness). A strict dependence of RBE on the age of irradiated gametes and also on dosage can be observed. In the first brood, ordinary X-rays were less efficient, the RBE was more than 1, but only at 1000 r. With 2000 r the RBE was lower than 1. From these very exact data we can conclude that comparison tests are only decisive when they are made with the same stage and the same dose, and under the same conditions.

In conclusion, we found four strictly marked stages of spermatogenesis with different reaction patterns. Stage 1 (0-3 days after treatment) is characterized by an indifference to different qualities of radiations. The same indifference is found to changes of milieu: absence of O_2 (in press);

treatment with cyanide, acid, or dihydroxydimethyl peroxide (Sobels, F. H., Nature 177: 979-982, 1956); and fractionation of dose (Muller, J. Genet. 40: 1, 1940). Stage 1 involves mature sperms. Stage 2 (5th-7th day) shows a marked difference between radiations of high and low energy and also an increased dependence on milieu. Stage 3 (8th-10th day) differentiates among all three types of radiations and is very dependent on milieu. During this period sperms are used which were probably irradiated at meiosis, as we conclude from our experiments with irradiated pupae. Finally, stage 4 (11th-13th day; irradiated spermatogonia) is again indifferent. It is not easy to explain this result. As in previous experiments (inducing lethal factors with 31-MeV X-rays, killing mice, killing Drosophila embryos, stopping mitosis; (Fritz-Niggli, Fortschr. Röntgenstr. 80: 28-38, 1954), we found mostly a higher efficiency of ordinary X-rays. But in stage 3 the 31-MeV X-rays, with a great spectrum of energy and lower energy than 30-MeV electrons, have the lowest efficiency. We assume that for stage 1 (mature sperms) OH and H radicals are effective and for stage 3 (meiosis) H_2O_2-molecules, the production of which is sensitive to milieu and to linear energy transfer 31-MeV X-rays (mean energy 10 MeV) and 30-MeV electrons may have a small difference in linear energy transfer.

We hope that these experiments which have been started will furnish some solutions to the problem. The assistance of Miss H. Inauen and the measurements of 30-MeV-electrons by Dr. Sempert are gratefully acknowledged.

(Table 1 and Table 2 on opposite page.)

Table 1. Embryonic lethality in %. Each value represents the mean of six experiments with a total of 3000 eggs.

Relation	Days between irradiation and insemination												
	Brood I			Brood II				Brood III			Brood IV		
	1	2	3	4	5	6	7	8	9	10	11	12	13
30-MeV electrons/910 rad	17.4	14.6	30.7	20.8	20.9	31.2	31.4	58.9	42.8	36.1	16.5	10.1	9.1
180-keV/930 rad	16.8	16.3	16.0	11.0	43.6	49.1	52.4	75.5	69.0	52.3	6.4	5.0	4.3
31-MeV X-rays/950 rad	17.3	13.9	28.6	19.9	19.0	33.9	34.6	22.3	22.3	19.3	10.8	7.9	7.2

Table 2. RBE in different days between irradiation and insemination

Relation	Days between irradiation and insemination												
	Brood I			Brood II				Brood III			Brood IV		
	1	2	3	4	5	6	7	8	9	10	11	12	13
30-MeV electrons/180-keV X-rays	1.0	0.9	1.9	1.9	0.5	0.6	0.6	0.8	0.6	0.7	2.6	2.0	2.1
30-MeV electrons/31-MeV X-rays	1.0	1.1	1.1	1.1	1.1	0.9	0.9	2.6	1.9	1.9	1.5	1.3	1.3

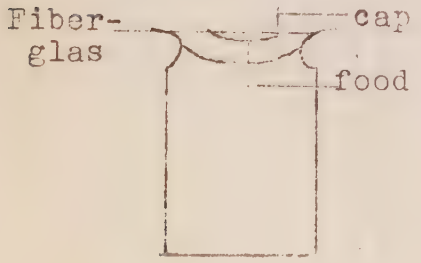
TECHNICAL NOTES

Cordeiro, A. R. Orcein with Janus Green B for better salivary-gland-chromosome staining. A simple but valuable improvement of the classical aceto-orcein staining of Drosophila polytenic chromosomes is attained by adding 30 mg. of Janus Green B for each 10 cc of aceto-orcein stain (orcein, 2 g; acetic acid, 60 cc; distilled water, 40 cc). The "Harleco" brand has been used. The dissection is made, as usual, in aceto-orcein only. The isolated glands or cerebellar ganglia are transferred to a drop of orcein with Janus Green B on a slide and then covered with a coverslide. The smear is made by the common technique. Advantages: The chromosomes are strongly stained by the dye mixture, and the cytoplasmic material remains lighter than it usually does with the aceto-orcein method. Ageing of the aceto-orcein-Janus-Green-B smears improves instead of reducing the contrast.

Fuscaldo, Kathryn E. A technique for the collection of bacteriologically sterile flies. A method has been devised for the sterilization of large numbers of eggs of Drosophila for the purpose of maintaining stocks of sterile flies. The method makes available the largest number of sterile eggs with a minimum of loss due to handling injuries, arrested development from chemical injuries, or mechanical loss through sterilization procedures.

Virgin males and females are collected and maintained separately for 4 days on cornmeal-molasses-agar medium. The flies are mated on the fifth day, using approximately two males to one female, for 24 hours. On the sixth day the females are separated and placed on fresh food for about 6 hours before egg laying, to allow for recovery from the effects of etherization.

The collection of eggs is accomplished as follows. A square, 2" x 2", of finely woven Fiberglas cloth is placed over a slice of solid medium about 1" square, which is put on the under surface of a half-pint-bottle cap. The cap is then inserted into the mouth of a half-pint milk bottle so that the surface of the Fiberglas is presented to the flies. The food seeded with living yeast serves to induce the flies to lay their eggs on the surface of the cloth, which is of sufficiently close weave to prevent the eggs from falling through to the food. The fertilized females are placed in the bottle and allowed to lay eggs for 12 hours, after which the eggs are collected by removing the cloth containing them and securely tying the ends with cotton thread, making a small egg sac. This egg-containing sac can then be put through the sterilization procedure.



The sac is immersed in a 3% solution of Clorox for twenty minutes to dechorionate the eggs. After dechorionation, the eggs are rinsed in sterile insect Ringer's solution and then placed in a solution of Roccal, 1:10,000, for sterilization. This step takes about one hour. The egg sac is rinsed again in sterile Ringer's solution for 10 minutes (3 changes). After sterilization the sac is held by the bound ends over the mouth of a bottle of

sterile culture medium. It is then cut just below the binding thread and allowed to fall open onto the food. The larvae can then crawl into the food upon hatching. The sterilization procedure is carried out in a sterile transfer box equipped with three UV lamps. After 7-8 days of incubation, before eclosion of the flies, the sterility of each culture is tested by inoculating two slants of each of the following media: TGE, Sabourandes dextrose, and wort agar. Those culture bottles whose inocula produce growth on the agar slants are discarded as being contaminated.

Sterile cultures can be maintained indefinitely by subsequent transfer of adults to fresh, sterile medium. Such transfer is accomplished by shaking into new bottles in the sterile transfer box. The sterile culture medium is the standard cornmeal-molasses-agar medium without living yeast, to which 1 ml of folic acid (.036 g/100 ml 20% alcohol) has been added to each bottle after pouring. The medium is rendered sterile after preparation by autoclaving for 20 minutes at 15 lbs. The necessity of the folic acid was indicated in previous experiments, since larvae raised on unsupplemented sterile medium failed to pupate or eclose.

(Supported by a research grant, C-2440, from the National Institutes of Health, administered by Allen S. Fox.)

Hochman, Benjamin. A new counting plate.

Originated in 1956 by Dr. W. W. Newby, this counting plate embodies all the favorable features and none of the disadvantages of glass, paper, and painted metal plates currently in use. It is constructed of white formica, 1/16" in thickness. A sheet of formica measuring one square foot can be purchased at a builders' materials company for about one dollar. Employing a fine-toothed blade in a power saw, pieces of desired size can be cut. We find 3" x 5" makes a satisfactory plate size. The cut edges are smoothed with sandpaper. The smooth, hard formica surface provides an excellent background for examination of flies. Moreover, the surface is unaffected by ether or alcohol, is very resistant to needle scratching, and can be cleaned easily with soap and water. Unbreakable under normal laboratory conditions, some of these plates have had constant use for over a year without showing signs of wear. For reasons of economy these formica counting plates could be utilized profitably in genetics laboratory courses as well as by individual investigators.

Hollingsworth, M. J. A simple device for ensuring that Drosophila bottles contain an equal amount of food.

The spout is removed from a large polythene funnel and replaced by a glass tube made by removing the bottom of a 3" x 1" vial. The tube is fixed to the funnel with melted polythene from the spout. The flow of food is controlled by two valves made from a length of glass rod and two rubber bungs. The upper bung fits snugly into the neck of the funnel when the rod is pushed down. The lower bung is tapered to ensure a good fit when the rod is lifted up. The rate of flow of the medium is controlled by adjusting the two bungs. Lateral play is eliminated by means of wire supports across the mouth of the funnel.

Jaeger, Celso P., and Jaeger, Euterpe C. Chemically defined medium for D. willistoni.

A chemically defined medium for D. willistoni was prepared by altering the Hinton et al. (1951) medium for D. melanogaster.

	mg/ml
L-Arginine	0.559
L-Cysteine	0.480
L-Glutamic acid	4.418
Glycine	1.745
L-Histidine	0.484
L-Isoleucine	1.260
L-Leucine	2.345
L-Lysine	1.337
DL-Methionine	0.339
L-Phenylalanine	1.008
DL-Threonine	0.756
L-Tryptophan	1.745
L-Valine	1.355
Fructose	7.5
Cholesterol	0.3
Ribonucleic acid	4.0

	µg/ml
Biotin	0.020
Calcium pantothenate	16.0
Choline chloride	575.0
Pteroyl glutamic folic acid	3.0
Pyridoxine	2.5
Riboflavin	10.0
Thiamine	2.0
Niacinamide	12.0

	mg/ml
KH_2PO_4	1.83
NaH_2PO_4	1.89
$NaHCO_3$	1.4
Agar	20.0

Meyer, Helen U. Obtaining completely relaxed and stretched live larvae of D. melanogaster.

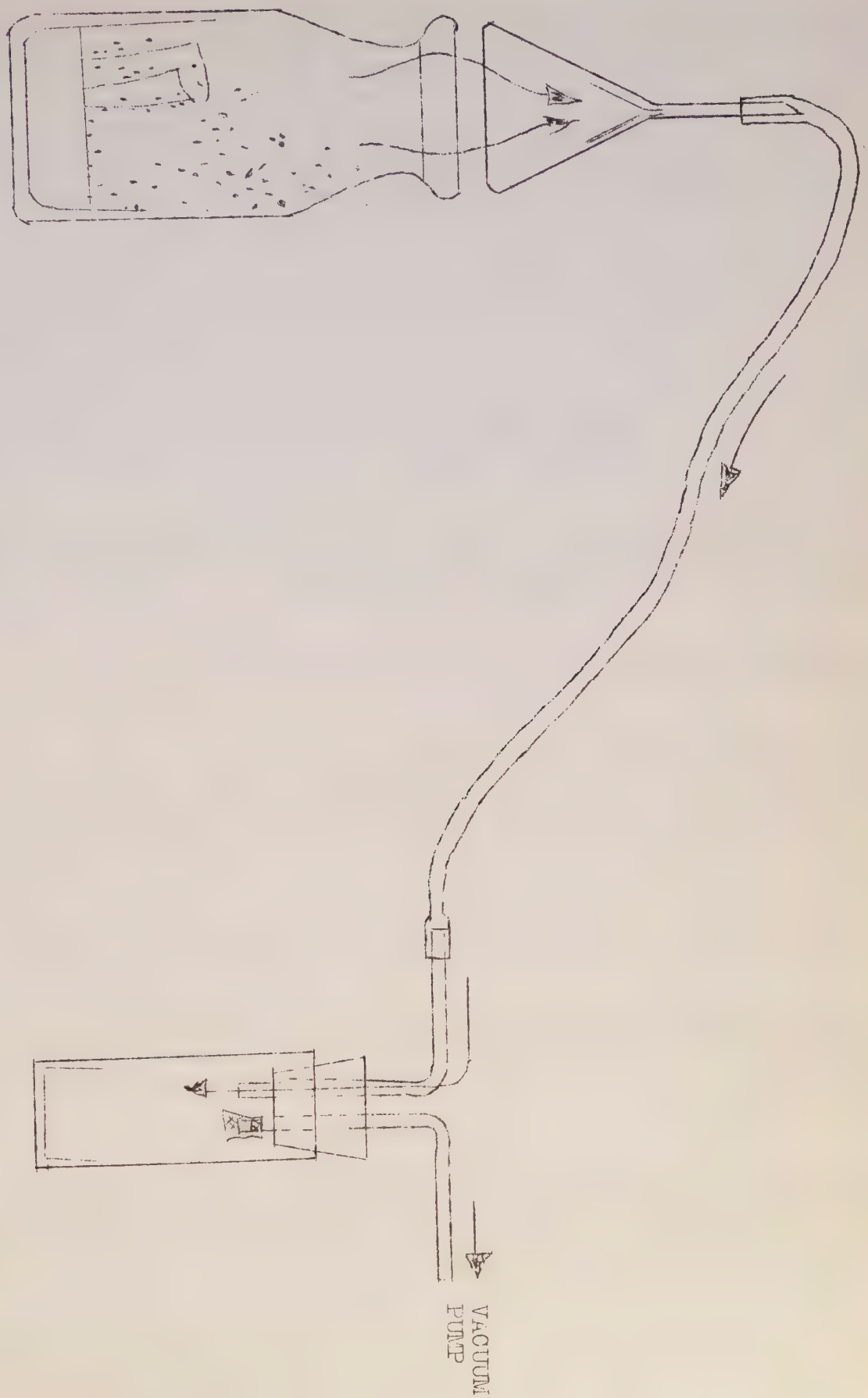
It is sometimes desirable to obtain completely stretched-out and relaxed larvae for better microscopic observation in vivo or for other purposes. This can be accomplished by placing young larvae, in a drop of water, into a container filled with nitrogen gas. After about 5 minutes the larvae have lost their muscle tone completely, they are limp and stretched out at full length. When brought back into air after this period, they regain their muscle tone, start the muscular contractions again, and resume their normal shape, apparently without any ill effects.

Nicoletti, B. A method for counting a large number of flies easily.

For counting a large number of flies from extremely crowded population bottles, when it is not necessary to consider the sex of the individuals, the following system has been used. The etherized Drosophila are placed and uniformly distributed on the surface of a sheet (11.7 x 8.3 inches) of sensitized paper (Agfa Copyright). The paper with the flies is conveniently exposed and a positive photocopy is made (photoprint Dupleca, white light 150 volts/20 seconds). It is possible to detect the exact number of flies by the dark spots on the positive paper. The sheets are used for counting individuals, checking each mark with a colored pencil. On each sheet about 600-700 flies can be counted.

Nicoletti, B. System for collecting flies from population bottles.

The system shown in the drawing has been used successfully for collecting flies from extremely crowded population bottles. A funnel is placed on


VACUUM
PUMP

the opening of the bottle and the flies are pulled into it by gentle impulses from the vacuum pump. The sucking produced by the vacuum carries the animals into the etherizing bottle. This prevents the standard medium from running and individuals from escaping. After the mass of the population has been transferred in this way, the funnel is replaced by a small glass tube, so that the few flies left walking on the sides of the bottle or on the paper can be collected. This latter arrangement is also useful for collecting etherized individuals without using forceps, saving a considerable amount of time.

MATERIALS REQUESTED OR AVAILABLE

A. R. Cordeiro requests the favor of receiving any available back issues of DIS. Address: Instituto de Ciências Naturais, Universidade do Rio Grande do Sul, Avenido Paulo Gama, Pôrto Alegre, Brazil.

W. M. Hexter (Department of Biology, Amherst College, Amherst, Mass.) would like a duplication covering the singed locus of D. melanogaster.

R. C. King is interested in obtaining stocks of female-sterile mutants other than those currently available at Northwestern (dor, sc^{S1}, sta, dm, sn^{1}, sn^{2}, sn^{3}, sn^{4}, sn^{5}, sn^{34e}, sn^{36a}, sn^{50k}, oc, gg^{2}, lz^{s}, ras^{4}, ty, na, r^{9}, fu, fu^{ff}, fu^{57a}, fu^{57f}, ds^{38k}, ds^{52k}, fes, rn, ap^{4}, ap^{56f}, fs2.1, cg, nw^{2}, mi, mr, cmp, bf, and sv^{de}).

E. Ortiz (Laboratorio de Citogenetica, Serrano 113, Madrid 6, Spain) would like to receive reprints that are available, and back issues of DIS.

Henry L. Plaine (Zoology Department, Ohio State University, Columbus) still desires to be notified of or to receive any new suppressors found in D. melanogaster. He would also like to be notified of any suppressors found in other species.

Duplicate copies of DIS numbers 3, 9, 11, 13, and 15 are available at the Department of Biological Sciences, Stanford University, Stanford, California. Laboratories requiring these particular issues to complete sets may inquire of Professor David Perkins.

PUBLICATION AND NOMENCLATURE

Report of K. Brehme-Warren

The second edition of "The Mutants of Drosophila melanogaster" by Bridges and Brehme has been in preparation since March, 1957, and is well under way. The author has an extended leave of absence from college responsibilities and will be able to work full time on this volume until September, 1958. Investigators are urged again to send new material, and particularly corrections and new information on mutants already described in the 1944 edition and subsequent numbers of DIS. The deadline for such material is February, 1958.

Nomenclature: A report has been received from Dr. Ernst Hadorn of the meeting held in Zurich in August, 1957, of the International Committee on Genetic Symbols and Nomenclature. Only one departure has been made from the symbols used in the 1944 edition of Bridges and Brehme: enhancers are to be written En- or en- (examples: En-S, en-N^8) instead of the shorter symbols E- or e- previously in use. This change will be adopted in the second edition. As no new terminology has been suggested for pseudoalleles, these will be described under their original names (examples: bx, Cbx; S, ast; w, w^a), and their pseudoallelic relationships will be indicated in the descriptions and on the maps.

ANNOUNCEMENTS

André Dreyfus Foundation. Announcement of the International Genetics Prize for 1958.

In accordance with its statutes and regulations the Board of Directors of the Foundation André Dreyfus invites interested persons to register as applicants for the International Genetics Prize of 1958.

1. The International Genetics Prize for 1958 amounts to Cruzeiros 150,000 (one hundred and fifty thousand).

2. The prize is open to individual scientists or groups of research workers from any country, working on problems of genetics or related fields.

3. The International Genetics Prize is intended for the promotion of: (a) the development of research programs; (b) travel for purposes of research; (c) publication of the results in research or of monographic summaries.

4. Applications should be accompanied by: (a) the candidate's

curriculum vitae; (b) a list of publications; (c) a detailed plan of the research program proposed or a copy of the manuscript for publication. Note: In the case of a team of research workers, the application should be signed by one of its components.

5. In case of equality of qualifications, preference will be given to the project which may have more direct influence on the development of genetical research in Brazil.

6. Applications accompanied by supporting documents should be received by the Secretary General of the Foundation at the address below not later than the 31st of January, 1958. (Jenny Dreyfus, Secretária Geral da Fundacao-Premio André Dreyfus, Rua Belfort Roxo 40, apto. 502, Copacabana, Rio de Janeiro, D.F.)

Creighton, Harriet B. A request from the Travel Assistance Committee for the Genetics Congress. A number of geneticists will be coming to the International Genetics Congress in Montreal in August, 1958. Undoubtedly, some of them can come before the Congress and some can stay after the Congress. Undoubtedly, also, some would like to visit laboratories in the United States, but to do so they will need dollars. The Travel Assistance Committee for the Congress is anxious to know which laboratories would like to help foreign geneticists by inviting one or more either to give a lecture or to come as a consultant on research. If you have any funds for lectures or for consultations which you could allocate to geneticists, would you let the Committee know as soon as possible. (Harriet B. Creighton, Department of Botany & Bacteriology, Wellesley College, Wellesley 81, Mass.)

Following is a list of geneticists the Committee expects will be in Montreal:

Professor Ivar Johannson, genetics in animal breeding (animal husbandry, particularly cattle)
Dr. H. P. Donald, genetics in animal breeding (cattle breeding, particularly monozygotic twins)
Dr. Francois Jacob, Institut Pasteur
Dr. Alan Robertson, genetics in animal breeding (quantitative inheritance, both in animal husbandry and in Drosophila)
Professor Hans Nachtsheim, genetics in animal breeding (genetics of rodents, particularly hereditary abnormalities from a comparative and development point of view)
Dr. C. Syrach Larsen, cytogenetics and plant breeding (forest genetics)
Dr. D. Lewis, cytogenetics and plant breeding (self-incompatibility, particularly in fruit trees, but also in other organisms)
Professor H. Kihara, cytogenetics and plant breeding (wheat genetics and fruit cytogenetics)
Professor H. Stubbe, mutation and mutagenesis (mutations, both radiation-induced and spontaneous)
Dr. J. R. S. Fincham, physiological genetics (gene-enzyme relations in Neurospora)
Dr. E. Hadorn, physiological genetics (development and biochemical genetics, particularly in Drosophila)
Dr. P. Michaelis, physiological genetics (cytoplasmic inheritance in Epilobium)

Dr. N. P. Dubinin, genetics in evolution (evolutionary genetics in Drosophila)
Dr. C. Pavan, physiological genetics in Drosophila and physiology of the salivary gland
Dr. H. Harris (human biochemistry)
Professor R. Ceppellini (human blood groups)
Dr. Maurice Lamy (twin studies)
Dr. Cavelli-Sforza (evolutionary genetics)
Dr. Jen Book (human genetics)
Dr. Arne Müntzing (plant genetics)

Herskowitz, Irwin H. Proposed Drosophila Research Stock Center. Plans for a Drosophila Research Stock Center in the Department of Biology at Saint Louis University are under way. It will aim not only to maintain present and future stocks but to encourage their use in research. It is hoped to start the Center about July, 1958. Each worker will be notified by mail several months before the start as to how the Center will operate initially.

To help determine the desirability, scope, and mode of operation of the Center, your comments on the following plans would be appreciated.

1. The Center will be restricted, at least at first, to maintaining stocks of D. melanogaster.

2. Each stock sent to the Center for keeping will need to be described by the sender as to genotype, phenotype, and research purposes for which it is especially useful.

3. Workers sending the Center more than 10 stocks in any week will have to clear the sending date in advance with the Director.

4. A fee of $5 is to be charged for each stock sent to the Center. Such a stock will be maintained for a minimum of 5 years before the Director can remove it from the stocks held, unless permission to discard the stock is granted by the worker who originally sent it in. The fee is intended to discourage flooding the Center with stocks of limited or questionable research value.

5. Stocks sent to the Center become the property thereof, and are available to all bona fide research workers. No stock may be withdrawn from the Center's stock holdings without the approval of the Director. However, he would welcome comments from any worker on the suitability of maintenance of a stock.

6. Only stocks whose mutant phenotypes are easily defined and whose mutations are localized are to be accepted for keeping.

7. Stocks which are difficult to maintain because they cannot be cultured on standard brewers' yeast-enriched cornmeal-molasses-agar medium at a temperature of 17° C are not acceptable.

8. On the day of receipt of a stock the Director will mail a post-card notice to the sender. Within two months of receipt, a notification of acceptance or rejection for maintenance will be mailed by the Director. If not accepted, $4.50 will be refunded for each stock.

9. Any number of stocks may be obtained from the Center, as frequently as requested, by any researcher. For each stock requested, 25¢ in U.S. stamps or money must be provided.

10. A yearly list of stocks and their most desirable uses will be made by the Center, to be distributed as a part of "Drosophila Information Service" or as a separate sent together with it, or mailed independently. Workers listed in DIS will receive this listing free.

11. The Center is not designed to be a competitor of private companies which sell stocks for classroom work or nonresearch purposes.

12. The Center, once established, is planned to be partly self-supporting.

A stock sent to the Center may continue to be maintained by the sender. Thus the Center will provide a second place for maintenance as insurance against loss of valuable stocks. Or laboratories that wish to eliminate the keeping of certain stocks--for space, budget, or personnel reasons--may do so, yet be assured that they will be maintained for at least five years. Duplication of stocks maintained by various laboratories can be eliminated in some cases.

Space is available at Saint Louis University for maintaining approximately 4000 stocks.

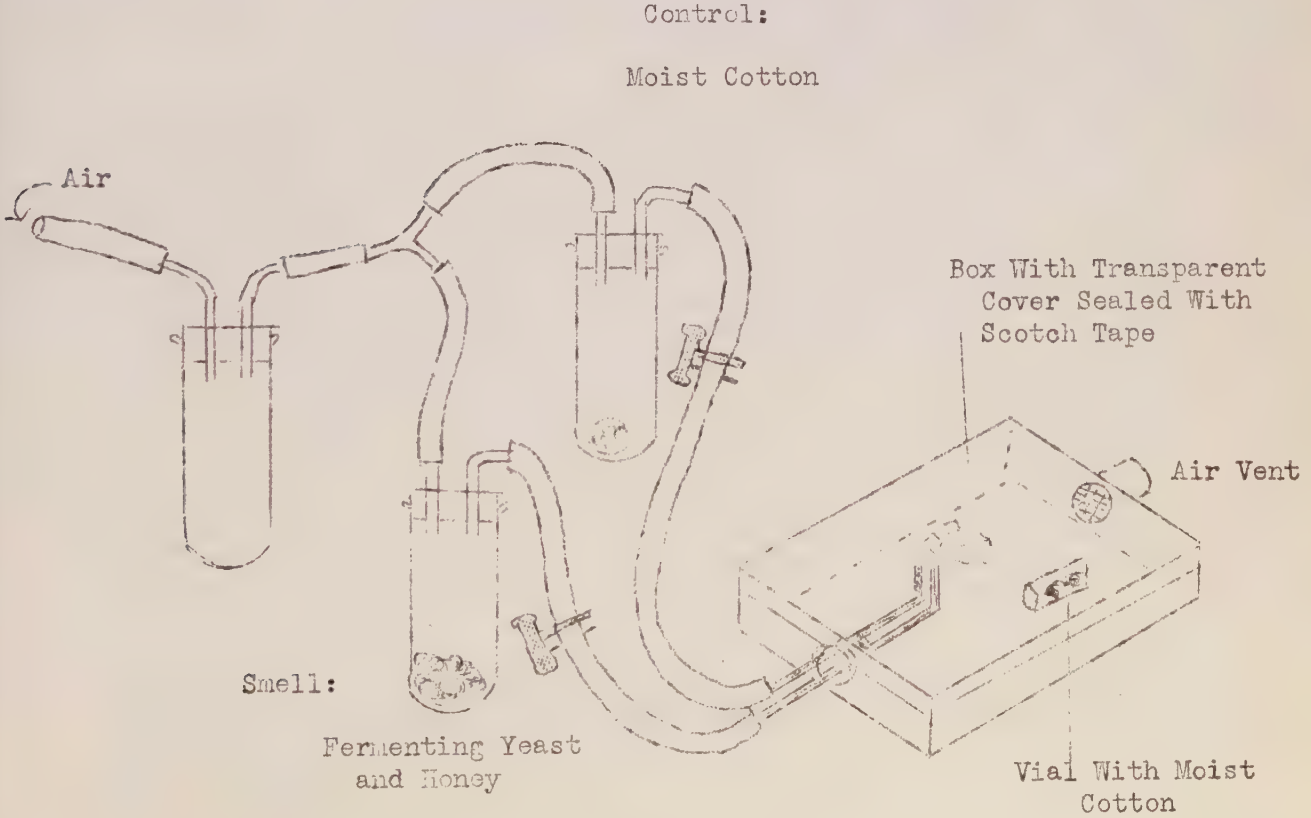
TEACHING NOTES

Hexter, W. M. A two-factor sex-linked cross involving gene interaction.

The mutants g^{53d} and w^a, both sex-linked recessives 42.9 map units apart, are phenotypically indistinguishable. The phenotype of these mutants is orange, varying somewhat with age. The class is given as parents females of one mutant and males of the other, and the cross is designated simply as "orange 1 x orange 2." F_1 females are wild type and F_1 males are orange. F_1 are interbred to raise an F_2. The F_2 females are expected to be wild type and orange in equal frequencies, and the F_2 males theoretically should be approximately 60 per cent orange (parental types) and 40 per cent crossovers, half of which are wild type and half double mutant (w^a g^{53d}). Actual class data were: females, 1530 wild type, 1402 orange; males, 690 wild type, 1420 orange, 454 white. Deviations from equality were due primarily to differential viability of the various genotypes. The student is confronted with the following facts: a wild-type F_1 female; a mutant F_1 male; a new phenotype (white) in the F_2 but confined to one sex. From this information the student should conclude that orange 1 and orange 2 are not alleles and are recessive; that one of them is sex-linked; and that white is probably due to the combined action of orange 1 and orange 2. The student then usually assumes a second gene that is autosomal. This assumption will not account for the data. The second gene is then assumed also to be sex-linked, and the conditions of the problem

are satisfied if linkage is assumed to be about 45 per cent. In addition to the unexpected phenotypes and the challenging yet not too complicated analysis, this experiment has the advantage of simple and rapid classification.

Rizki, M. T. M. Genetics of behavior. We have been doing some experiments on the response of normal and mutant strains of D. melanogaster to the smell of food, in order to demonstrate the possible influence of inheritance on the behavior pattern of flies. A simple apparatus constructed from a cardboard box with a transparent cover is sufficient to carry out these experiments (see drawing). Air saturated with the desired mixture is blown through rubber tubing into the box. Flies are generally starved overnight and conditioned in the plastic-covered box, which contains a small vial of moist cotton. The flies will respond to the control of moist air if water is not available during the conditioning period. Dessication is also avoided. When air saturated with the odor of yeast and honey is blown into the box, the following components of behavior of Ore-R flies can be observed in an orderly sequence: (a) fluttering of wings, (b) shaking of abdomen, (c) looping or circling, (d) walking straight to the orifice of the tube which is the origin of the odor. This experiment can be modified by introducing other variables, such as different kinds of smells and different mutants. Students have found these experiments interesting and instructive, particularly those who are interested in psychology and behavior.



RESEARCH NOTES*

Abrahamson, S. Oxygen depletion and viability.

In the course of X-ray experiments concerned with the effects of oxygen concentration on chromosome breakage in Drosophila oöcytes (Abrahamson, 1956), it became important to learn how long females could be maintained in an environment devoid of oxygen. Females were placed in sealed chambers containing nitrogen (obtained from Linde "hi-purity" cylinders) for periods of 1, 3, and 18 hours. After the nitrogen exposure the flies were observed for four days. Better than 95% survival was obtained in the first two groups; the group confined for eighteen hours suffered 100% mortality.

Altenburg, Luolin S., and Edgar Altenburg. Absence of detectable mutagenic effect of sodium formate, ethyl acetate, or Fremy's salt when administered to the polar-cap cells of D. melanogaster.

A number of compounds have been tested for mutagenicity in this laboratory by exposing the pole cells of developing D. melanogaster eggs to the chemicals and testing the survivors for recessive lethals in the second pair of autosomes by Muller's "sifter" technique. This report will be confined to certain of the chemicals, shown in the table below, which have so far yielded mutation rates as low as (or lower than) the control rates usually obtained with this material (between 0.3% and 0.7% in different experiments). Each dosage was sufficient to cause noticeable mortality of the eggs.

Agent	Chemical Formula	Conc.	No. ♂♂ Tested	No. Chroms. Tested	No. Rec. Lethals	Mutation Rate
Sodium formate	HCOONa	1 M	40	1266	4	0.3±0.2%
Ethyl acetate	$CH_3COOC_2H_5$	100% vapor for 20 min.	29	1006	2	0.2±0.1%
Fremy's salt	$^{\cdot}O\text{-}N(SO_3K)_2$	10^{-2} M	52	933	1	0.1±0.1%

Sodium formate and ethyl acetate appear to be nonmutagenic, in contrast to ethyl formate vapor, which produced a mutation rate of 4.7±1.6% (Rec. Genet. Soc. 25: 632, 1956). Fremy's salt is a strong oxidizing agent, which in water dissociates into moderately stable free radicals. The mutation rate of 0.1±0.1% produced by this compound is significantly below the control rate, but this apparent lowering of the rate below the spontaneous level must so far be accepted only tentatively, inasmuch as the rates being dealt with are so small. A fuller account of these experiments will appear elsewhere. (This work has been supported by a grant from the American Cancer Society for work of Edgar Altenburg and associates.)

Annan, Murvel E. Effects of degree of desiccation on X-ray-induced egg mortality in Drosophila females.

Virgin D. melanogaster (Oregon-R) females were exposed in a desiccation chamber for periods of 0, 6, 12, or 18 hours immediately before irradiation with 5000 r X-rays. The X-rays were provided by the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor. Immediately after irradiation, the females were placed

*An asterisk before the title of a note indicates that the author has given unrestricted consent for its citation in publications.

with males (3 males to 2 females) in egg-collecting chambers. The eggs were collected every 12 hours for 108 hours, were counted, and were cultured at 25° C. Failure to hatch was the criterion of egg mortality. The percentages of egg mortality for the nonirradiated females were subtracted from the corresponding percentages for the irradiated groups. The remainder was divided by the percentage of eggs hatched in that nonirradiated group, and the result was multiplied by 100 to give the percentage of X-ray-induced egg mortality (after Herskowitz, Genetics, 1957). These values are given in the table below.

Hours of desiccation	Hours after treatment								
	12	24	36	48	60	72	84	96	108
0	72	-36	14	12	13	6	2	- 4	- 6
6	100	73	75	80	76	62	73	83	81
12	-80	- 6	15	-20	-11	-12	- 8	-10	- 7
18	83	86	25	60	64	58	58	50	28

A minus figure indicates that eggs from the X-rayed females concerned had a lower mortality than did those from nonirradiated females in the same desiccation treatment group. The variability obtained for the first 36 hours may have been due, in part, to variability in fertilization. The periods from 48 hours on are believed to establish a reproducible pattern of X-ray-induced egg mortality characteristic for the degree of desiccation. The difference between induced egg mortality with 12 hours of desiccation and that with either 6 or 18 hours is the most significant aspect of the results. (This work was supported by a U.S.P.H.S. research grant.)

Baglioni, C. Studies of ommochrome biosynthesis in D. melanogaster.

The mutant lightoid was found to block ommochrome synthesis, like v, cn, and st. By crossing ltd with the mutant brown, which completely blocks the synthesis of the red pteridinic pigments, a white-eyed strain was obtained (bw ltd) which is almost identical in phenotype to the strains v bw, cn bw and st bw. An attempt was made to localize the metabolic block in ltd by means of feeding experiments. Larvae of the tested strains were grown on a medium containing kynurenine or 3-OH-kynurenine. As a control, v flies were cultured under the same conditions. At emergence ltd flies showed no brown pigment, whereas the v flies were normally pigmented. It seems that the ltd metabolic block involves a reaction affecting 3-OH-kynurenine or some 3-OH-kynurenine derivative. In feeding experiments ltd cannot be distinguished from st or cd flies, which also do not become normally pigmented when given kynurenine or 3-OH-kynurenine.

In order to reveal the accumulation of different unmetabolized compounds in these strains, a chromatographic analysis of tryptophan metabolites was made by the two-dimensional paper chromatographic method of Dalgleish (Bioch. J. 64: 481, 1956). Preliminary results indicated that kynurenine and 3-OH-kynurenine are always present in the two-dimensional chromatograms of ltd, st, and cd strains in larger amounts than in the wild type. In the chromatograms of cd or st flies a pale blue fluorescent spot was found, which was not found in v, cn, or ltd chromatograms. This spot was present in the wild type, but at a lower concentration than in cd or st flies. We shall refer to the substance of this spot as the ltd^+ substance.

Attempts aimed at identifying the ltd$^+$ substance, by making grams of ltd fly extracts mixed with some known tryptophan metab showed an overlapping of the ltd$^+$ spot with the spot given by a synthetic xanthurenic acid. In other solvents, better suited fo separation of xanthurenic acid derivatives (like methanol, butan water, ammonia in the proportions 40:20:20:20:1, or butanol saturated with 0.2 M ammonia), it was possible to separate the ltd+ substance from added xanthurenic acid. Further work will be necessary to get more precise details about the chemical structure of the ltd$^+$ substance.

Baglioni, C. Two new pteridins, found in D. melanogaster. Research of Viscontini and his collaborators showed that the red pigment of Drosophila is made of at least three components: drosopterin, isodrosopterin, and neodrosopterin, which were isolated and chemically defined. Our aim was to find a simple paper chromatographic method which would allow separation of the pteridinic compounds without any previous chemical treatment of the flies. The best resolution of the pteridins was obtained by two-dimensional chromatography, using n-butanol-acetic acid-water (4:1:5) as solvent for the first run and KCl 20% for the second run. It was possible to separate quite well the compounds isolated by Viscontini and to demonstrate the presence of two undescribed pteridins, which have the same Rf values as drosopterin and isodrosopterin in the first run, but may be differentiated from them in the second run. Since these pteridins have the same fluorescence color and absorption maxima as drosopterin and isodrosopterin, we propose the names paradrosopterin and paraisodrosopterin (as suggested by Professor Hadorn). The amount of these five components of the red pigment was determined in 30 of the commonest eye-color mutants; the concentration of the pteridins was estimated fluorimetrically.

Previous results indicate that drosopterin, isodrosopterin, and neodrosopterin, even though reduced in many mutants, nevertheless maintain a constant relative ratio. Paradrosopterin and paraisodrosopterin seem to behave in an independent manner, not maintaining a constant ratio with the other pteridins. An extreme situation was observed in the mutant sed, which has no paradorsopterin or paraisodrosopterin. Although extractions were made with a large amount of flies, and a concentrated solution of the pigment was chromatographed, no trace of these two substances was found. Further studies of the metabolic significance of this finding are now in progress.

Bart, Carol. A retest of the frequency of spontaneous loss of the yellow$^+$ region of the scute-8 chromosome. Because of the far higher rate of spontaneous loss of the region (containing y$^+$) adjacent to the left end of the scute-8 chromosome in some of the experiments reported by Belgovsky (1938, 1939) and in those by Sidorov (1941) and by Lindsley (1955) than in those by Frye (1957, 1958), and because of the importance of the control (spontaneous) rate in studies of radiation-dose dependence, further tests of the spontaneous frequency were carried out on the stock used by Frye. As before sc^8 B males were mated to y w In49 f virgins. The males were 0-24 hours old, the females 48-72 hours old, when first mated. After 48 hours with these females (lot a) in vials containing 5 flies of each sex, the males were separated out and mated to a second lot of females (b) for another 48 hours, again in groups of 5 of each sex. Both a and b females were put through 5 broods of 48 hours each. The offspring scored were as follows:

with r coll 25°	Regular B ♀	yellow B ♀	Regular y w f ♂	Non-dis. y w f ♀	Non-dis. B ♂
From lot a	13,532	1 1/2	13,229	11	3
From lot b	20,203	1	19,550	29	18

The y B ♀ designated as 1/2 was a mosaic that did not transmit the mutant yellow phenotype of the sc^8 B chromosome. There was in addition a forked-mosaic B ♀, which failed to transmit the new forked, and a non-Bar female that transmitted the phenotype without lethality. Counting only the transmitted yellows, the total is 2 among 33,740 disjunctionally produced daughters or about 1 in 17,000. This result is not significantly different from that of 7 yellows among 263,794, or about 1 in 38,000, found by Frye, although 4 of Frye's yellows proved to have attached X's. Both yellows found here had separate X's, and their y sc^8 B X proved lethal to males. The present low control rate is also like that found for scute-Sl by Lüning (1952) and for scute-8 in one of Belgovsky's experiments.

On the basis of Sidorov's analyses, confirmed by those of Lindsley, we assume that in all probability our two exceptional yellows resulted from exchange between Y^S and the left heterochromatic region of the scute-8 chromosome. It seems likely that differences in the amount and structure of the heterochromatin would occur not uncommonly both in scute-8 and in Y chromosomes and would affect the frequency of their interchange.

(This work was supported by a grant to H. J. Muller and associates from the U. S. Atomic Energy Commission, Contract AT(11-1)-195.)

Barzilay, Roy. Tumor phenocopy produced by cold. Larvae of several Drosophila strains were exposed for 24 hours to 5° C ± 1 in a refrigerator, at different stages of their development. Many of the treated larvae developed dark masses, resembling the so-called "melanotic tumors." The work described here was carried out with strain D/118 of D. melanogaster (D balancing a lethal extracted from a local population) and D. simulans (see table). Positive results were also obtained with a number of other strains.

		Non-tumorous	Tumorous	S	Tumor Incidence
D/118	Treated	1474	647	2121	30.4%
	Control	494	22	516	4.3%
D. simulans	Treated	552	108	660	16.4%
	Control	1155	-	1155	-

In D/118, the highest tumor incidence was scored in individuals treated during the 60th to the 84th hour of larval life. This "sensitive period" includes the stage which according to Rizki (1957) is characterized by a peak in the frequency of lamellocytes in the hemolymph. The participation of the lamellocytes in the formation of tumorous aggregates was observed both by light and phase microscopy, thus confirming Rizki's conclusions.

Bateman, A. J. Mutations in irradiated spermatocytes. References to the radiation responses of premeiotic male germ cells are usually concerned with spermatogonia, though spermatocytes, which also belong to this category, are likely to have very different responses. In order to isolate the various stages of spermatogenesis, the classical brood pattern based on 3-day mating periods is too coarse, as 3 broods will include all the stages from spermatogonia onwards. A study of crossing over between b pr and vg in males irradiated with 1000 r and mated each day to 2-3 females has been started. This mating rate is adequate to prevent mixture of sperm maturing on successive days. Results so far are as follows (missing days were unsampled):

Days from irradiation	2	5	6	7	8	9	10	11
Crossovers	0	0	6	7	3	7	13	0
Noncrossovers	1651	1836	1400	1452	687	1540	2313	1990
% crossovers	0.00	0.00	0.43	0.48	0.44	0.45	0.56	0.00

Concurrent tests for egg-hatching and deleted X's confirmed earlier experiments (Bateman, 1956, 1957), indicating that the same day corresponded to the same germ-cell stage throughout. Day 8 consistently showed a drop in fertility (shortage of sperm), which is presumed to be due to the lethal action of X-rays on late spermatogonia. Then days 6 and 7, and possibly 8, will yield the products of irradiated spermatocytes. Some responses of spermatocytes compared with earlier (spermatogonia) and later (spermatids) stages:

Dominant lethals: gonia, nil; cytes, very high; tids high
Deleted X's: gonia, nil; cytes, very high; tids low
Induced c-o: gonia and cytes equal; tids nil

If a broad classification of germ cells according to their response to mutagens is to be made, it should be into spermatogonial and post-spermatogonial rather than into pre- and post-meiotic.

Baumiller, R. C. The pre-adult viability of spontaneous mutations in D. melanogaster. In order to obtain an accurate estimate of mutation frequency when mutants can be scored only as adults, the pre-adult viability of mutants compared with normals must be taken into account in an environment where the mutant is represented by one individual existing among a preponderance of individuals that are normal (i.e., nonmutant and otherwise identical), since this is the circumstance under which mutants occur. Accordingly, pre-adult viabilities were determined in both the "Maxy" stock and the same stock in which spontaneous mutations had recently occurred. The mutants y^{A241}, f^{585}, w^{568}, w^{A659}, and w^{ch} were each compared to the parental "Maxy" stock normal for these genes. The stocks were kindly supplied by Dr. A. Schalet.

Normal and mutant flies were placed each in their own dacron-net-enclosed cylinder, which was placed netting-down on individual petri dishes containing nutrient medium seeded with yeast. After a 24-hour egg-laying period parents were removed; the plates were incubated for another 24 hours, when larvae were picked and placed in vials containing yeast-seeded standard medium. One hundred normal larvae and 1 mutant larva were transferred into each vial. (Ratios of 300:1 and 10:1 were also tested. The ratio 300:1

showed a lower percentage of survival for both the normal and mutant flies than was found in the tests using the ratio 100:1, but the comparative rate of survival was approximately the same. Thus pre-adult viability differences were shown not to be an effect of crowding nor to be grossly affected by it. Therefore, the use of a lower ratio was indicated as more economical. The ratio 10:1 also gave about the same comparative rate of survival, but the wide variance in numbers surviving in individual vials made this ratio unsatisfactory. Thus the ratio 100:1 was used exclusively.)

The results are shown in the table.

Normal:mutant::100:1

	Mutant	No. vials	% normal emerging (%A)	% mutants emerging (%B)	%B/%A	P
1	w^{A659}	19	60.8±1.14	79.0± 9.41	1.30	.05
2	y^{A241}	20	70.0±1.04	80.0± 8.91	1.14	.27
3	w^{ch}	20	72.3±1.03	85.0± 7.89	1.17	.12
4	w^{568}	20	73.1±0.98	95.0± 4.87	1.30	.0001
5	f^{585}	20	83.9±0.82	40.0±10.90	0.48	.0001

The sex ratios for emerging normal and mutant flies were similar, females outnumbering males as is expected in the "Maxy" stock. Tests are listed in the table in the order in which they were done. From the percentage of normal adults, it is evident that as the experiment proceeded the technique improved. This, however, does not affect the validity of the conclusions to be drawn, since the percentage of mutants emerging improved concomitantly. In the case of y^{A241} and w^{ch} the results suggest a higher relative pre-adult viability than in normal flies, but the differences are not significant. On the other hand, w^{A659} and w^{568} both show the same high rate of comparative larval viability (1.30), although the separate tests do not have the same level of significance. The difference between w^{568} and normal flies is highly significant, whereas the difference in the experiment using w^{A659} is barely significant because of the small number of adults obtained. The f^{585} stock showed a significantly lower relative pre-adult viability (0.48). In all cases where the rate of pre-adult viability favored the mutant, it was found that mutants were generally among the first group of flies to hatch. The forked mutant, however, in all but one test hatched out at least one day after the earliest group.

The data confirm that there are mutations which, though considered to be detrimental to the over-all productivity of the mutants, are actually supravital at least during the larval stage of their life cycle. It is also shown that in the "Maxy" stock the mutation frequency from w^+ to w might be overestimated by a factor of 0.3, and the frequency of mutation of f^+ to f might be underestimated by a factor of about 2 if relative larval viability were not taken into account. The viability differences found between individual mutant and normal flies in either the positive or negative direction, depending on the mutant tested, demonstrate the necessity for making approximate viability allowances when calculating actual, as opposed to observed, spontaneous (or induced) mutation rates. The results do suggest, however, that adult-determined mutation rates are probably wrong by no more than a factor of 2 or so.

(This work was supported by a grant to Dr. I. H. Herskowitz from the Atomic Energy Commission, Contract AT-11-1-633.)

Brehme-Warren, K. Valuation or rank of mutants. In preparing the second edition of "The Mutants of Drosophila melanogaster" by C. B. Bridges and K. S. Brehme, a new set of criteria for ranking the mutants has been devised. The older system, printed on page 8 of the 1944 edition, worked well while Bridges made most of the judgments. Since his death, however, it has proved to be so subjective as to give variable results in the hands of different investigators. Accordingly, at a seminar in the Genetics Department of Indiana University in February, 1958, a new and more objective system was worked out, with the assistance of Drs. H. J. Muller, I. I. Oster, E. A. Carlson, and H. U. Meyer. It reads as follows:

Valuation or Rank. Rank 1. A mutant allowing sharp classification; of good viability and fertility in both sexes in the condition (heterozygous, homozygous, or hemizygous) in which it is used for classification; and accurately located.

Rank 2. A mutant less good in any one of the above characteristics, but still of considerable use as a marker.

Rank 3. A mutant faulty in any two of the above characteristics but still retaining enough classifiability to be useful.

Rank 4. Poor markers.

The letter A is added to indicate association with a chromosome aberration. If a mutant has been insufficiently studied, it should not be given a rank.

At the request of the author and through the courtesy of Drs. O. G. and M. B. Fahmy, more than 75 new mutants from the Fahmy laboratory have been assigned rank by Dr. I. I. Oster, and the results, ranging from RK1 to RK4, have shown the new system to be easily applicable. The author is applying it to both new and old mutants.

Brosseau, G. E., Jr. Crossing over between Y chromosomes in male Drosophila. Results of experiments with sterile Y chromosomes indicated that exchange between two Y's (or an X-Y^{arm} and a Y) takes place with a higher frequency than has been reported for other types of exchange in the male. To test this possibility, males carrying two marked Y chromosomes were tested for recombination. Males of the constitution y v/YB^{S}/sc^{8}.Y:bw^{+}; bw were crossed to y v/y v females. Recombinant sons were recovered in the progeny of two males. One of these was a single y v/Y:bw^{+} son; in the other case there were 4 y v/Y:bw^{+} sons and a single y v/YB^{S}/Y:bw^{+} son. In this latter case a recombinant Y and a nonrecombinant Y were recovered in the same gamete, therefore the exchange must have occurred at the four-strand stage. In other experiments, recombinants between a sterile Y chromosome (a sc^{8}.Y chromosome with an induced deletion in the fertility region of the long arm) and Y^{L} of Y^{S}X.Y^{L} have been recovered in comparable frequencies.

The frequency of Y/Y recombinants is about 1 in 2000-3000, whereas the frequency of exchange between the X and Y chromosomes in the male is about 10^{-4} and the frequency of recombination between autosomes is even less. Several clusters have been recovered as well as the simultaneous recovery of reciprocal recombination from the same male, indicating that the exchanges are gonial. The process of recombination between Y chromosomes is under further investigation.

One of the recombinant Y chromosomes recovered from males carrying the

two marked Y's mentioned above is a potentially useful Y. This Y has B^S at the end of the long arm and y^+ (sc^8 tip) at the end of the short arm; thus both arms of the Y are terminally marked. It should be very useful in such studies as detachment of attached-X's and translocations involving the Y.

Brosseau, G. E., Jr., and D. L. Lindsley. A dominantly marked Y chromosome: YB^S.

The available marked Y chromosomes carry as duplications normal alleles of sex-linked or autosomal genes, and their utility relies on the use of crosses carrying the appropriate recessives. An exception to this is the Hw effect of sc^8.Y. The desire for a Y chromosome marked with a readily classifiable dominant gene has led to the construction of a Y with its long arm marked terminally with B^S.

From a cross of $T(1;4)B^S$, B^S car/$XY^L.Y^S$, y^2 su-w^a w^a (bb?) $Y^L.Y^S$ females by XY, y cv v/0 males we recovered B^S car^+ sons. These males could have been the product of exchange between w^a and the $T(1;4)B^S$ breakpoint followed by nondisjunction of X centromeres, and consequently $XY^L.Y^S/B^S/0$ in constitution. Such males are sterile owing to the B^S duplication. Alternatively they could have been the product of exchange between the $T(1;4)B^S$ breakpoint and car, and $X^D.B^S$ $Y^L.Y^S/0$ in constitution, X^D and B^S representing the distal and proximal segments of $T(1;4)B^S$ respectively. Such males are fertile. All recovered B^S males were crossed to y v bb/0 females, and several fertile cultures resulted; they yielded y v bb daughters, y v B^S daughters, and fertile B^S sons which were $X^D./B^S$ $Y^L.Y^S$ in constitution. These males were irradiated in an attempt to delete enough of the euchromatin of B^S $Y^L.Y^S$ to allow males carrying the resultant derivative in addition to a normal X to be fertile. The irradiated males were crossed to free-X females; the two classes of sons produced normally were X/0 and X/B^S $Y^L.Y^S$, both sterile. The only fertile sons would be those carrying the desired derivative chromosome and the $X^D./B^S$ $Y^L.Y^S$ sons resulting from maternal nondisjunction. Consequently, the progenies of the above mating were transferred to fresh medium and fertile cultures were recovered. The desired product was recovered from one such fertile culture. Since YB^S carries the tip of the B^S duplication it must also carry the distal part of chromosome 4 translocated to the X by $T(1;4)B^S$; it is consequently thought to be of the following constitution: 4^D B^S (bb?) $Y^L.bb^+$ Y^S. This YB^S chromosome is characterized by good viability and normal disjunction from an X chromosome in the male. The B^S is a strong Bar, and both males and females carrying it show a narrow Bar phenotype.

Burdette, Walter J. Stability of tumor incidence in Drosophila after injection of mammary tumor agent.

Numerous viruses are known to be carried by insect vectors. Recently the polyoma virus known to be associated with tumors in mammals has been found to cause tumors of multiple tissues in different species, and the incidence of inherited tumors in Drosophila has been altered by the introduction of susceptibility genes into cytoplasm carrying the genoid for CO_2 sensitivity. In order to test whether the milk agent affects the appearance of tumors in Drosophila, a preparation of the virus from C3H mice was injected into females of the tu vg bw, tu^{36a}, and Oregon-R strains. Tumor incidence in the progeny was then compared to that in parallel control cultures. The results may be summarized as follows:

Strain	tu vg bw				tu^{36a}				Oregon-R	
	Injected		Control		Injected		Control		Injected	
	No.	%	No.	%	No.	%	No.	%	No.	%
Males	3242/3245	99.9	604/608	99.3	56/1775	3.2	15/491	3.1	2/7572	0.03
Females	3565/3575	99.7	546/546	100.0	77/1810	4.3	49/566	8.7	1/7518	0.01
Total	6807/6820	99.8	1150/1154	99.7	133/3538	3.7	64/1059	6.0	3/15090	0.02

This evidence does not suggest that the injections increased the incidence of tumors. Whether the negative results are due to failure of the virus to propagate or to its being inert with reference to tumorigenesis in Drosophila has not been determined.

Carlson, Elof A. Variegated position effect at the dumpy locus in D. melanogaster.

Several induced mutations at the dumpy locus (dp--2, 13.0) were produced with high doses (4000 r) of X-rays in Oregon-R wild-type males which were mated to echinoid dumpy·clot (ed dp cl) females. A small number of these (about 10%) showed considerable asymmetry of wing and thorax effects. Therefore these variegated mutants have been designated dp^{W}, dumpy-warped. Most were homozygous lethal, although occasionally a few homozygotes were obtained with an extremely weak and deformed phenotype, showing dull variegation of the eyes, necrosis of the thorax, and club-like wings. There were three which only showed the variegation in the male offspring, the females always remaining homozygous ed dp cl when testcrossed with males of the composition dp^{W}/ed dp cl, indicating Y-2 translocations. All together, ten have been obtained. Two are lost; one is an X-2 translocation; three are 2-3 translocations; and the remaining exception has not yet been analyzed, although it too prevents crossing over in the ed-cl region. A sample of these were tested with an extra Y chromosome, using the stock X.Y y v; ed dp cl. The variegated position effect was completely suppressed in all tested cases. When individuals heterozygous for the rearrangement and the tester stock were inbred, the F_2 homozygotes, genotypically X.Y y v; R(dp^{W}), were almost completely normal. At a warmer temperature (28° C) an occasional slight vortex or mild oblique wing may be expressed in a few of these homozygotes. The variegated position effect does not extend to the outside markers ed (11.0) and cl (16.5). Since the dumpy region also controls a lethal factor, it is possible that the position effect is also affecting this character. However, with dp^{T} (olv^{m}) the compounds with dp^{W} are similar to the homozygotes but are somewhat more viable. Stocks of these variegated mutants may be kept without too much difficulty when dp^{txI} Cy is used as a balancer. The compound produces a peculair effect on the Curly wings, which become opaque and deformed, probably as a consequence of the weak oblique effect exerted by dp^{txI} (lv^{i}). No effect is observed for the Curly when the balancer does not contain the dp^{txI}.

These typical variegated position-effect mutants are of interest primarily because the dumpy series is located in a complex locus with at least seven separable mutant sites which can express numerous pleiotropic effects.

Usually the wing and thorax expressions go together, although in at least one-third of the cases the variegation for the right and left sides may be expressed independently. No case has been found yet among the induced exceptions in which variegation affects exclusively the thorax or the wing. Only one of these exceptions is moderately expressed; the rest are indistinguishable in phenotype from one another. Several other induced mutants at the dp locus involve rearrangements, but no variegation is present. Some of these may represent intra-locus (perhaps inter-sublocus) breaks, and others euchromatic position effects of breaks outside the dp region. The total induced mutation rate is high (about 1/300) at this dose. Tests are being carried out to determine if the variegated types can, in some cases, represent intra-locus breaks.

(These mutants were obtained during a series of experiments at Indiana University which were partially supported by grants to Dr. H. J. Muller and associates from the U.S. Atomic Energy Commission, Contract AT(11-1)-195. Their analysis is part of a project sponsored by the National Research Council of Canada, Annual Grant No. A 776.)

Castiglioni, M. C. A new stock with lethal pseudotumors in D. melanogaster.

The stock tu-mwh (tumor-multiple wing hairs) shows pupal lethality. Nearly 88% of the dying pupae carry pseudotumors, and show, at the same time, abnormalities in the organs originating from the imaginal discs. Examinations in toto and on microtime sections of larvae at different ages revealed irregularities in the disc folds and also alterations within the lymph gland, which clearly is transformed into a melanotic mass. In extreme cases there is also an excess of hemolymph fluid, so that swollen larvae are produced, which are also larger than the normal ones. Invasion of hemolymph cells has never been observed; therefore the cause of lethality is probably complete degeneration of the lymph gland.

Castiglioni, M. C. Cell melanization induced in larvae of D. melanogaster.

When larvae are treated in Bouin fixative solution at a temperature of 60° C for 60 seconds, dark points and spots appear in the body. The dark points are nearly black and correspond to two types of blood cells, referred to in my papers as small and mid-sized cells. The spots are larger than the points but not so dark; they correspond to large cells. Response to the treatment is different in different stocks (wild stocks-Varese, S. Maria, Valdagno, Gaiano, Moltrasio; tumorous stocks-tu A_2 , tu B_3 , e 144 melanotic). The difference consists in the percentage of blackening larvae, and in the type and distribution of points or spots. The type depends on the composition of the hemolymph formula (frequency of the different cell types), so that with this technique it is possible to reveal the cytological composition of the lymph gland. Patterns of distribution show greater or lesser uniformity in different parts of the body. The melanization of the large cells parallels somewhat the effect of the tu genes of tumorous stocks.

Cooper, K. W. A probable heterochromatic deficiency in $In(1)sc^{L8}$, the approximate location of bobbed, and the size of block A.

In large neuroblasts, the heterochromatic region of the prophase X chromosome consists of the small right limb (XR) followed by four nearly equal-sized

heterochromatic segments (hA, hB, hC, and hD). Each of these heterochromatic segments is roughly the size of a fourth chromosome, and together they make up the whole of the heterochromatic region (Xh) of the large, left limb of X, with the nucleolus organizer lying between the second and third respectively. The structure of the heterochromatic half of X, then, from left to right, is: hD, hC, NO, hB, hA, sfa, XR.

In the case of In(1)sc^{L8}, the rightmost break of the inverted piece lies in hB, and a large series of prophase figures shows that only half or so of hB is inverted. The remaining, uninverted, proximal heterochromatic length, however, is reduced in size, and it is very likely that a segment approximately half the size of hB (or perhaps somewhat more, including a distal piece of hA) has been lost from the chromosome. In(1)sc^{L8}, therefore, very likely arose as a three-break event, and possesses a deficiency to the right of the nucleolus organizer which, at present, contains no known genes. The proximal break of this deficiency very likely lies close to, or at, the proximal break of the deficiency in In(1)bb^{-}.

Inasmuch as the rightmost break of the inverted piece of the bobbed-deficient In(1)bb^{-} lies in hC, and as bobbed is inverted by In(1)sc^{L8}, it follows that bobbed is close to the nucleolus organizer and either in the proximal half of hC or the distal half of hB; that is, bobbed lies in the mid sector of Xh, and not close to the junction of the euchromatic and heterochromatic regions as generally believed. Furthermore, since block A is defined as a distal portion of the length of Xh that lies between the most proximal breaks of Ins(1)sc^{L8} and sc^{8}, it follows that the region between the proximal breaks of the inverted segments of Ins(1)sc^{L8} and sc^{8} possesses at least one breakable site (namely, that giving the deficiency-sc^{L8}), and that block A itself must be considerably smaller in size than the morphological element hA.

Crowell, Villa B. Experiment to determine percentage of larval-pupal death due to X-irradiation of P_1 males.

Despite some earlier reports that dominant lethal effects found in F_1 zygotes derived from irradiated spermatozoa are confined to the egg stage, some mortality in the larval-pupal period is to be expected on the ground that the great majority of mutations are detrimental and have some dominance. This question was investigated by comparing the mortality, in this period, of zygotes derived from irradiated and control males that had been obtained from the same Oregon-R stock, divided randomly into the treated and control groups, and crossed to unirradiated y t^2 v f virgins. The irradiated males were given 4000 r 3 to 3 1/2 days after eclosion, were at once mated for 22 1/2 hours to a first lot of females, whose offspring were discarded, and then remated to a second lot for 7 1/2 to 8 hours. The larvae that hatched from eggs laid during a 4-to-6-hour period by the females of the second lot were gently collected on needles, 24 to 32 hours after the beginning of the egg laying; and another collection, of the later-hatching larvae, was made some 40 to 42 hours after the beginning of the egg laying. The larvae were counted, scored as to color (yellow mouth parts here indicating males), and carefully placed in fresh, uncrowded culture vials. The same procedure was followed with the control series.

Because of complications arising from the possibility of sometimes mistaking the color of mouth parts, the results for both sexes will be reported collectively here. Somewhat more than 1700 control larvae and 2700 larvae from treated fathers were placed in vials. Survival to maturity

fluctuated between about 90% and 95% among the controls of the six different experimental series, and was usually distinctly lower in the corresponding treated lots. To avoid as far as possible the bias that might be caused by determinate cultural differences between series in which the ratios of control to treated larvae might also differ, use was made of the harmonic mean method of statistical analysis developed by Muller (1941, Amer. Nat. 75: 264-271). The results showed that approximately 6% (with a standard error of not more than 1%) of the just-hatched larvae were prevented from reaching maturity by dominant detrimental effects of the radiation given to the paternal spermatozoa. This result is in the range of our expectation based on previous work on dominance and on the frequency of lethal and detrimental mutations.

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Di Pasquale, A., and Santibanez S. Koref Pseudotumors in D. melanogaster and D. simulans.

Flies were collected at Palermo, Sicily, in August and October, 1957. From the first collection, 16 D. melanogaster and 46 D. simulans were kept, and from the second collection, 21 melanogaster and 75 simulans females. Each female was cultured singly, and lines were established by taking 4 pairs at random from each of the following generations to constitute the subsequent one. Pseudotumor incidence was recorded each generation for 12 generations.

In the first generation, over-all tumor incidence was as follows: D. melanogaster, 1st collection, 4.6%; D. melanogaster, 2nd collection, 1.9%; D. simulans, 1st collection, 2.7%; D. simulans, 2nd collection, 9.6%. In D. melanogaster in the 1st collection, 81.2% of the lines presented tumors, against 47.6% in the second collection. In D. simulans, 85.4% and 87.8% of the lines of the 1st and 2nd collections, respectively, showed the trait.

By the third generation, all but two of the lines of D. simulans from the August collection had presented the tumoral trait. Up to the twelfth generation, the character was not lost in any of the lines, and its incidence remained on the whole constant. An attempt to study the genetic factors responsible for the trait revealed them to be partially dominant in D. simulans. In D. melanogaster, however, as has been previously described, they were recessive.

These results seem to us to indicate the following: (1) Pseudotumors are relatively frequent in the natural populations of D. melanogaster and D. simulans studied. (2) There seem to be seasonal differences in the manifestation of the trait. (3) The genetic factors responsible for it are present in practically all the individuals captured. (4) There seems to be a difference in the physiological mechanisms by which the phenotypically similar trait is manifested in the two sibling species studied. (5) It is suggested that the factors responsible for the pseudotumors may confer certain adaptive advantages on their carriers, so that natural selection favors them. Seasonal differences may also be attributed to the plasticity of the genetic constitution, which may be responsible for differential responses in accordance with environmental pressures. Further studies are under way to clarify these hypotheses.

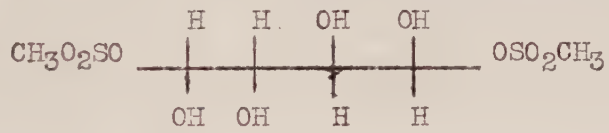
Doane, W. W. Meiosis in unfertilized eggs of D. melanogaster.

Young unfertilized eggs laid by virgin females of an Oregon-R strain of D. melanogaster were sectioned and analyzed. Cytological details studied in 93 such eggs indicated that in every case meiosis had gone to completion, contrary to views expressed in the literature (e.g., Huettner, J. Morph. 39, 1924; Sonnenblick, Biology of Drosophila, 1950). In twenty of these eggs, four interphase or prophase nuclei were found and identified as the products of maturation. The remaining eggs contained one, two, or three groups of chromosomes whose appearance resembled that of the polar-body chromosomal groups described by Rabinowitz (j. Morph. 69, 1941) in fertilized eggs. The sequence of events following maturation varies somewhat in different eggs, depending on which of the meiotic products fuse with one another. Details of the events following maturation will be described elsewhere.

Fahmy, O. G., and Myrtle J. Fahmy A nontoxic mutagenic sulphonate in D. melanogaster.

It has been shown (DIS-25; J. Genet. 54, 1956; Nature 180, 1957) that the alkyl-methanesulphonates, both mono- and difunctional as regards the methanesulphonoxy ($-OSO_2CH_3$) group, are mutagenic when injected intra-abdominally into adult male Drosophila. The over-all activity of difunctional compounds of this series on the postmeiotic germ cells was found to vary according to the size and molecular configuration of the alkyl moeity of the molecule, in between the active groups.

Recently a compound of the above series, in which the central moeity is is the sugar mannitol, has been tested for mutagenicity.



1:6-dimethanesulphonoxy mannitol.

This compound is highly soluble in water and completely nontoxic to the injected flies, even at doses more than 10 times the highest tolerated dose of the least toxic of the other dimethanesulphonates. In spite of this low toxicity the mannitol derivative possesses decisive mutagenic activity. An injected concentration of 8×10^{-2} M (corresponding to a dose of 2.4×10^{-8} Mol. per male) induces an average of 5.6 ± 0.5% sex-linked recessive lethals among postmeiotic sperm. Like the rest of the alkyl-methanesulphonates, the mannitol derivative has very low activity on the premeiotic stages of the germ line.

Farnsworth, M. W. Quantitative studies of DNA in wild-type and Minute larvae.

One aspect of the over-all problem of delayed growth and development characteristic of Minute larvae is DNA production. In order to rule out the possibility that lag in DNA synthesis is a major factor in the delayed development of these mutants, estimates of DNA content and concentration in homogenates of wild-type and Minute larvae of D. melanogaster have been made.

Methods of isolation of nucleic acids were those of Schmidt, Thannhauser, and Schneider. Estimation of DNA in µg/ml of final extract was carried out

according to Ceriotti's microchemical method, employing the indole reaction (Ceriotii, J.B.C. 198: 297, 1952). Control values were obtained from 15 separate determinations on homogenized Canton wild-type larvae. For each determination, from 200 to 800 72-hour larvae were used, the average number being around 450. Experimental stocks were M(2)1^2 and M(3)w heterozygous larvae. Nine determinations were carried out with M(2)1^2, and seven with M(3)w. The number of larvae used per determination was approximately the same as with control material.

All data were calculated as (1) µg DNA/ml of final extract (concentration), and (2) µg DNA/larva (content). On the basis of these calculations, the following data were obtained:

Canton wild-type

Concentration:	0.3 ± .02	µg/ml
Content:	0.7 ± .04	µg/larva

Minute (2) 1^2

Concentration:	0.3 ± .03	µg/ml
Content:	0.4 ± .05	µg/larva

Minute (3) w

Concentration:	0.4 ± .08	µg/ml
Content:	0.3 ± .08	µg/larva

From these data it is obvious that there is no significant difference in DNA concentration in the three stocks tested. The lower values for DNA content per larva in the Minutes as compared to wild type undoubtedly reflect the somewhat smaller body size of the Minute larvae. This size difference is probably due to the smaller cell size of the Minutes. In general, the data indicate that delays in growth and development cannot be ascribed specifically to defects in DNA metabolism.

Forbes, Clifford. Marked-Y technique for the detection of primary nondisjunction. An improved method of detecting nondisjunction, which gives particularly reliable evidence concerning the origin of each exceptional female, makes use of tester males from a stock with marked Y chromosomes. Females homozygous for yellow and vermilion are mated to y w.Y^S/sc.Y^L males. These males carry the wild-type allele of yellow on Y^L. Expected females and males are yellow and not-yellow vermilion, respectively. Exceptional females are not-yellow vermilion, and exceptional males are yellow white.

Frye, Sara. More X-ray-induced forkeds found not suppressible by su^w-f. Tests of 12 forked mutants produced by X-raying spermatozoa in the male in experiments to ascertain the frequency of induction of yellow deficiencies in the scute-8 chromosome have shown that none of them is suppressed by Whittinghill's suppressor, su^w-f. This result is in agreement with findings reported by Green (1956) but it differs from the findings regarding the X-ray-produced mutant f^x (supposedly a sublocus deficiency), discussed by Muller, Oster, and Ehrlich in this and previous issues of DIS.

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Glassman, E. Further studies of the maroon-like (ma-l) and rosy (ry) eye-color mutants.

In DIS-31 (p. 121), it was reported that ma-l^+/ma-l females had a maternal effect on their ma-l progeny, whereas a similar effect of ry^+/ry on ry progeny did not occur. These two mutants, although on separate chromosomes, are related in that both lack xanthine dehydrogenase, and accumulate excess amounts of this enzyme's substrates (hypoxanthine and 2-amino-4-hydroxy-pteridine) because the conversions to uric acid and isoxanthopterin do not occur. The simplest explanation of why ma-l is maternally affected is that these flies can utilize a maternal substance, whose synthesis they cannot carry out because of the genetic block. The reason ry is not maternally affected is that these flies are probably blocked in a biochemical reaction concerned with utilization of the maternal substance. This assumption is supported by the fact that females homozygous for ry can still exert a maternal effect on ma-l, indicating that ry flies can synthesize the necessary maternal substance for ma-l progeny.

Biochemical studies have now shown that the maternally affected ma-l progeny differ from typical ma-l flies in that they have normal amounts of red eye pigments and exhibit traces of xanthine dehydrogenase, and its reaction produces uric acid and isoxanthopterin. Whether the enzyme or some necessary factor for activity is the maternal substance cannot be determined; but it is also possible that it is the enzyme-forming system which is involved, mainly because the maternal effect persists to the adult stage, and proteins or simple activators might not be expected to persist that long without new synthesis.

The genetic localization of ma-l must now be reconsidered. It is obvious that the maternal effect (which diminishes as the bottle gets older) will interfere with crossover data unless progeny testing is carried out. Through the use of sn, m, f, and Bx in various combinations with ma-l, it was possible to relocate ma-l to the right of Bx on the sex chromosome, and not near v as reported in Bridges and Brehme.

Grell, E. H. The autonomy of reciprocal eye transplants between pn and K-pn.

Prune-killer is a third-chromosome dominant which in combination with prune causes second-instar larvae to die and has no other known mutant phenotype (Sturtevant, GENETICS 41: 118-123, 1956). Prune is an eye-color mutant which affects the pteridine pigments. It seems reasonable that K-pn might cause a further modification in the amounts of pteridines if the combination were not lethal.

In an attempt to circumvent the lethality and learn something about the interaction between K-pn and pn, eye discs were reciprocally transplanted between K-pn and pn larvae. The transplanted eyes were dissected out of the adult hosts, examined for color and chromatographed on filter paper in propanol-1:water:NH_4OH (60:30:1). No differences could be detected between pn eyes grown in K-pn or + hosts or between K-pn eyes grown in pn or + hosts. The pigmentation of the transplanted eyes was autonomous in all cases and the experiments did not give any indication of an effect of K-pn on pteridines.

Therefore the interaction between pn and K-pn is autonomous; and an effect on pteridines, if it exists, is expressed only when pn and K-pn are in the same cell.

Grell, Rhoda F. The effect of X-chromosome loss on variegation.

The cross of a v; bw male which carried an extra Y chromosome to a v; bw^{VDel}/ Sm1, al^2 Cy sp^2 female yielded one non-Curly gynandromorph. The left portion of the thorax of this fly was presumably female, since the prothoracic leg carried no sex comb and the wing on this side was considerably longer than the right wing. The right side of the thorax was male-like, for a normal sex comb was present on the first leg. The genital apparatus and pigmentation of the abdomen were also male.

The eye on the left side of the head was vermilion, and since the wings were not Cy it was assumed that the brown-variegation of this tissue was suppressed by the presence of an extra Y. The genotype of this tissue was interpreted as v/v/Y; bw^{VDel}/bw. The eye on the right or male side showed a white background with a few specks of color typical of unsuppressed brown-variegation in combination with vermilion. The genotype of this tissue was interpreted as v; Y; bw^{VDel}/bw.

The gynandromorph must have arisen through the loss of an X chromosome from an XXY female. That the unsuppressed condition did not result from the simultaneous loss of an X and Y chromosome was indicated by the fact that the fly was sufficiently fertile to produce four offspring. The X-chromosome loss also resulted in an unsuppressed brown-variegation. Thus it appears that an X chromosome is as effective as a Y chromosome in suppressing variegation.

Gruber, F. Frequency of heterozygous inversions in a natural population of D. immigrans from Israel.

The offspring of wild D. immigrans females, collected during two consecutive spring seasons at Qiryat 'Anavim (near Jerusalem), were examined cytologically for the types and frequencies of inversions. So far, only the long median inversion in the II L chromosome (inversion "A") has been found, with an over-all frequency of 33.9±4.7%. The rate of this inversion in the Q.'A. population is higher than that reported for Brazil (19.6±2.6%, Freire-Maia et al., 1953) and for Chile, where "A" exists side by side with 2 other inversions (Brncic, 1955). The data are summarized in the table.

Year	No. of chromosomes studied	No. of heterozygotes for inversion "A"
1957	45	17
1958	56	17
Total	101	34

Hillman, Ralph. Female sterility in singed^{55a}.

In a report of a new mutant in DIS-31 (1957), sn^{55a} was classified as a female-fertile allele at the singed locus. Recently, this mutant was used in a routine cross, where it was observed that homozygous sn^{55a} females did not lay eggs, but hemizygous males were fertile. The stock used at the time was y w^a cv sn^{55a} v/M5. Heterozygous

females from this stock were outcrossed to wild type, and the following homozygous chromosomes were recovered and tested.

Chromosome tested	Number	Fertility
y w^a cv sn^{55a} v	53	-
+ + + + +	14	+
y w^a cv + +	35	+
y w^a cv sn^{55a} +	33	-
+ + + sn^{55a} v	27	-
+ + + + v	26	+
y w^a cv + v	7	+
+ + + sn^{55a} +	4	-

Although the numbers involved were small and the map distances tested are 7 map units to the left and 12 map units to the right of singed, it appears that there has been the addition of a female-sterility factor associated with this singed allele. The stock has been sent to Dr. Harvey Bender at Northwestern University for further investigation.

Hoenigsberg, H. F., and Santibanez S. Koref. Courtship and mating preferences in D. melanogaster. By direct observation of the courtships of inbred and outbred lines of D. melanogaster from a common source, the authors were able to find the existence of discrimination and preference for their own type both in proximal and distant stimuli in inbred male choices.

Hoenigsberg, H. F., and G. P. Sironi. Chromatographic studies within the obscura group of species. One-dimensional paper chromatographic studies have been made with third-instar larvae and 48-144-hour adults of D. pseudoobscura, D. persimilis, and D. bifasciata. For comparative purposes, the same studies were extended to D. melanogaster. Preliminary results indicate that the third-instar larvae do not serve to distinguish pseudoobscura from persimilis. However, in 2-day-old male adults there is a marked quantitative difference as to intensity of fluorescence of the bluish and the yellow spots, Rf 0.353 and 0.411, respectively. In older flies (4 to 6 days) the differences between the two species are limited to the last blue-green fluorescent spot (kynurenin), which is considerably more intense in pseudoobscura than in persimilis females. D. bifasciata is different from pseudoobscura and persimilis. In third-instar larvae, D. bifasciata has greater fluorescence than the other two obscura species in the middle yellow spot, Rf 0.420. The second-day adult D. bifasciata lacks the uppermost pale spot of the series normally present in pseudoobscura and persimilis. D. melanogaster differs from the obscura species in the intensity of fluorescence of all compounds.

The authors believe that sibling species and species whose morphological phenotypes do not differentiate them from each other may reveal their differences at the physiological level. Therefore, as routine procedure, the

systematist may find chromatographic analysis helpful in identifying members of a species about which he does not feel certain. Such analytical information may serve to confirm taxonomic classifications, especially if developmental as well as the adult stages are chromatographed. From our experience we point out that although larval chromatograms did not discriminate between pseudoobscura and persimilis, chromatography of adults revealed the difference. D. bifasciata, on the other hand, was distinguished from the other two species by chromatographic patterns of third-instar larvae.

Horikawa, M. Tissue culture analysis of delayed lethal irradiation effect in D. melanogaster.

It is well known that the effect of radiation on Drosophila larvae is not their immediate death but a delay of pupation and a decrease in pupation and imagination rates, within a wide range of radiation doses. Furthermore, it was observed by the author that the body weight of irradiated larvae, whose pupation was delayed, not only increased but was considerably greater than that of control larvae. In the present investigation, the mechanism of this phenomenon was studied in some detail by the tissue culture method.

Wild strains (Oregon, Canton-S, Kochi, and Samarkand) and several eye-color mutants (bw, w, v, cn, v-bw, cn-bw, Bar, bar-3, and Dp/In(3L)P, In(3R)C, Sb e l(3)e of D. melanogaster were used as material. Third-instar larvae (about 80 hours old after hatching at 25° C), grown under sterile conditions, were irradiated with various doses of X-rays (160 Kvp, 25 mA, 1mm Al filter, 370 r/min, at a distance of 30 cm). They were dissected, and organs and tissues were removed under a binocular microscope in a sterilized glass chamber. They were cultured in a synthetic medium, and sensitivity to radiation was determined by observing their growth and differentiation. Some of the results obtained with a wild strain (Oregon) are shown in the table.

G=Growth, D=Differentiation, CC=Cephalic complex (ten bodies), N=Normal, I=Irradiated.

Organs and discs cultured	G and D rate	Doses (Kr) 0	0.5	1	3	5	10	15	20	25
N eye disc + ICC	G	+	+	+	+	+	+	+	±	-
	D	+	+	+	-	-	-	-	-	-
I eye disc + NCC	G	+	+	+	+	+	+	+	±	-
	D	+	+	+	+	+	+	+	±	-
I eye disc + ICC	G	+	+	+	+	+	+	+	-	-
	D	+	+	+	-	-	-	-	-	-
Culture medium	G	+		+			+		+	+
	D	+		+			+		+	+
Extracted metamorphic Hormone	G	+		+			+		+	+
	D	+		+			+		+	+

When irradiated (0-20 Kr) eye discs were cultured together with normal cephalic complexes (ten bodies), they showed pronounced growth and differentiation, as in normal eye discs. After high doses (25 Kr), however, neither growth nor differentiation was observed. When normal eye discs were cultured together with irradiated (3 Kr) cephalic complexes (ten bodies), the eye discs showed pronounced growth, but no differentiation. Since the cephalic complex

is known to exert hormonal control on growth and metamorphosis, it seems that the normal differentiation mechanism of organs and discs of the cephalic complex was virtually destroyed after low doses (3 Kr). Hence, one might suspect that the radiosensitivity of the bar-3 mutant, which showed the highest sensitivity in whole-body irradiation tests of the thirteen strains used, would be due to the higher sensitivity to radiation damage of the cephalic complex than the other strains. Furthermore, the metamorphic hormones already secreted from the cephalic complexes were not influenced even after high doses (25 Kr). The results of this experiment show that the delay in pupation and decrease in imagination rate are due to radiation damage of the cepahlic complex.

Imaizumi, T. Standardization of developmental stages in the Drosophila embryo. In order to facilitate studies of embryonic development, standard developmental stages have been established. The characteristics of embryos at different stages, as described below, can easily be recognized in dechorionated eggs through the transparent vitelline membrane. The standardization reported here was worked out with D. melanogaster and D. virilis. There is no essential difference between them as far as embryonic morphogenesis is concerned. Therefore the standards are probably applicable to other species of Drosophila.

Stage 1. Just after fertilization. Egg is filled with homogeneous protoplasm.

Stage 2. Egg contracted along the long axis, nuclei are multiplying interiorly.

Stage 3. Preblastodermic or blastema stage. A narrow zone of nucleated peripheral oöplasm differentiated from central yolk mass but not divided by cell walls. The stage is divided into two substages:

Substage 3a. Young blastema, in which pole cells are pushed out from the posterior surface.

Substage 3b. Late blastema, which is characterized by simultaneous multiplication of nuclei in the cortical zone.

Stage 4. Characterized by formation of the blastoderm, a unicellular layer on the surface of the embryo.

Stage 5. The stage during which both ventral and cephalic furrows are formed. It is divided into two substages in melanogaster and three in virilis:

Substage 5a. Early period. Secondary contraction of embryo is found only at the anterior part; in the meantime, the formation of ventral furrow takes place along the midventral line commencing anteriorly.

Substage 5b. Middle period, in which the ventral furrow extends onto the dorsal side beyond the posterior pole.

Substage 5c. Late period, in which the cephalic furrow is formed.

Substages 5a and 5b are distinct in virilis; in melanogaster the transformations at these stages occur almost simultaneously.

Stage 6. The stage in which a mass of pole cells moves toward the

center of the dorsal side; some transverse fissures (folds) appear at the same time.

Stage 7. Characterized by the formation of both proctodaeal and stomodaeal invaginations. Divided into two substages:

Substage 7a. Formation of proctodaeal invagination just completed.

Substage 7b. The stomodaeal invagination is formed; and at the same time the development of the germinal band formed by prolongation of the proctodaeal invagination advances into the interior of embryo.

Stage 8. The head and trunk are clearly distinguishable in the embryo.

Stage 9. The stage of preblastokinesis, preceding shortening of the embryo and dorsal closure. It may be distinguished from the next stage by the position of the yolk mass.

Stage 10. Involution of the head and dorsal closure take place. The posterior spiracles also are formed.

Stage 11. The stage at which segmentation of the body is evident.

Stage 12. This and the next three stages are identified especially by the shape of the midintestine. At this stage it is saclike and not coiled.

Stage 13. Coiling midintestine.

Stage 14. Ringed midintestine.

Stage 15. The stage before eruption of gas into the tracheae. The contents of the midintestine have become translucent and hardly visible through the body wall. There is also active movement of muscles.

Stage 16. The stage before hatching. The tracheae are filled with a sort of gas, which is not inhaled air but is produced inside the body.

Some characteristic photographs of the standard stages described above are now in press and will be published soon in Cytologia.

Imaizumi, T. The metabolic pattern of fluorescent substances in the development of Drosophila.

An attempt was made to demonstrate changes in patterns of fluorescent substances during the embryonic development of D. melanogaster, by means of two-dimensional paper chromatography. (Regarding other substances such as amino acids, sugars, and so on, reports will be made in the future.) More than 1000 dechorionated eggs were smashed directly on filter paper (Toyo-Roshi No. 50). The paper was then irrigated with hot water (75°-80° C) in a dark place. After drying, it was irrigated for a second time in the dark with the following mixtures: n-butanol, glacial acetic acid, and water (4:1:5 v/v, upper phase); n-butanol, n-propanol, water (2:2:1 v/v); n-butanol, 5 N acetic acid (2:1 v/v); n-propanol, 1% ammonia (2:1 v/v); phenol, n-butanol, water (160 g:30 cc:100 cc v/v); water saturated with isoamylalcohol; 5% Na_2HPO_4 aqueous solution; and collidine saturated with water.

The following fluorescent substances were found in the D. melanogaster embryo: kynurenine, 3-hydroxykynurenine, flavin-adenine-dinucleotide (FAD), flavin-mononucleotide (FMN), riboflavinyl glucoside (FG), two unknown flavin

compounds, probably new, and one unknown pteridine. Besides these components, a violet fluorescent spot was observed in place of kynurenine in the embryo of a vermilion strain. This may indicate the presence of an oxide of tryptophan. In brief, the fluorescent contents are rich in compounds derived from flavin throughout embryonic development, but very poor in pteridines. On the contrary, large amounts of pteridine compounds have been found, by the same method, in the embryo of Bombyx as well as in pupae of Drosophila. This is interesting and needs further investigation.

Changes in the fluorescent substances during embryonic development are tabulated below. The observations were made with 200 eggs per stage; the two-dimensional paper chromatography was performed by the irrigation with hot water the first time and with a mixture of butanol acetic acid and water the second time.

(1) Wild strain Oregon-R-S

Substances	Fl. color	Stages 1	2	4	7	9-10	13	14	16
Kynurenine	blue	++++	++++++	+++++	++++	+++	++	+	+(±)
3-OH kynurenine	green	-	-	-	-	+(±)	++	+++	++++
FAD	yellow	+	+	+	++	++	+++	+++	+++
FMN	yellow	+	+	+	+(±)	±	±	±	±
FG	yellowish	+	+	+	+	+(++)	++	++	++
Flavin compound I	yellowish	++	+	+	++	++	++	++	+++
Flavin compound II	yellowish	++	+++	+++	++	+	+(±)	-(±)	-
Pteridine	violet	+	+	+	+	+(±)	+(±)	+(±)	+(±)

(2) Eye-color mutants

Mutants	Substances	Stages 2	4	7	9-10	13	14	16
v	Kynurenine	-	-	-	-	-	-	-
	3-OH kyn.	-	-	-	-	-	-	-
	violet spot	++	++	++	+	±(-)	±(-)	±(-)
	Other components are similar to those in wild type.							
cn	Kynurenine	++++++	++++++	++++++	++++++	++++++	++++++	++++++
	3-OH kyn.	-	-	-	-	-	-	-
	Other components are similar to those in wild type.							
w	Kynurenine	++++++	++++	++++	+++	++	±	±(-)
	3-OH kyn.	-	-	-	±	+	++	+++
	Other components are similar to those in wild type.							

No remarkable difference could be found between the patterns of three mutants--st, se, and bw--and that of wild type. The tryptophan metabolism of the white mutant should be noted particularly; tryptophan is converted into kynurenine and further into 3-hydroxykynurenine in the embryonic stage

of white. This may indicate that no block exists, at least between tryptophan and 3-hydroxykynurenine, in this mutant.

The time of conversion of kynurenine into 3-hydroxykynurenine, or activation of kynurenine oxydase, may be learned by a comparison of tryptophan metabolism in the wild strain, vermilion, and cinnabar. The time of this conversion is at stages 9-10, the stage of involution of the head and dorsal closure, which corresponds to the stage of blastokinesis in the embryonic development of Bombyx. It seems that the conversion of tryptophan into kynurenine has already taken place in the ovary.

Details of this study will be published later.

*Jacobs, M. E. Influence of desiccation on tyrosine-oxidizing activity in D. melanogaster.

Previous studies (DIS-31) showed that desiccation of late larvae accelerated dopa-oxidizing activity. Further studies have shown that tyrosine-oxidizing activity was similarly increased after as little as one hour in a large air-tight chamber containing Dreirite, as compared with that of sibling larvae kept in a similar chamber containing moist gauze covered with wire cloth. Counts failed to show an increase in pupation rate accompanying the increase in tyrosinase activity, a fact that supports recent views of Dannell that melanization is not primarily related to puparium formation.

*Jacobs, M. E. Relation of age to sexual attraction in D. melanogaster.

Studies of ebony and non-ebony flies from a wild population in observation cells in light and diminished (red) light showed that non-ebony males more frequently mated with old (six-day) than with young (one-day) virgin non-ebony females; but with ebony females this difference was not noted. Males succeeded in mating with ebony females earlier than with non-ebony females; the latter showed avoidance behavior by kicking and bending down the abdomen. Males courted heads of one- and two-day-old females, in light or diminished light, more frequently than those of older or teneral females, and failed to court male heads only in diminished light.

*Jacobs, M. E., J. T. Bowman, and V. Walliser. Studies of a melanoma.

The stock of D. melanogaster used, herein named tu-55g, was discovered by the senior author in a wild population at Beaufort, North Carolina on July 28, 1955. Melanomas appear in the larvae, starting 55 hours after hatching. Every adult in a culture may show at least one melanoma, commonly on the abdomen, less commonly on the thorax, and rarely on the head. The largest larval melanoma is caudal, and this melanoma develops later than the others. The primary tumor chromosome is number two, as determined by use of dominant inversion marker stocks.

Kikkawa, H. Genetical analyses of resistance to parathion in a Swedish strain of D. melanogaster.

During a stay at the Biological Laboratory in Cold Spring Harbor during the summer of 1958, I obtained from Professor B. Rasmuson of Uppsala, Sweden a strain of D. melanogaster called "KSI," which was very resistant to parathion.

Genetical analyses have shown that the major gene for parathion resistance in this strain is also located at a locus near 65 on the second chromosome, as in Japanese strains like Hikone-R and WMB (see DIS-31, p. 125). Thus, resistance to parathion in D. melanogaster seems to be controlled by a single gene.

Kikkawa, H., and K. Abe. Genetic control of amylase in D. melanogaster. Amylase activity in larval, pupal, and adult stages of D. melanogaster varies in different strains. The gene responsible for strong amylase activity is semidominant, and probably located at 80± on the second chromosome. This gene seems to be concerned with amylase function in both digestive organs and body fluids.

King, R. C. Further studies of oögenesis in D. melanogaster. Oregon-R wild-type females (0-1 hours old) were fed for various lengths of time on medium containing 5-aminouracil (an analogue of both uracil and thymine). The medium contained 2% agar, 2% brewers' yeast, 0.5% sucrose, 0.5% propionic acid, and 0.1% of the analogue. Ovaries from flies fed 5 days on 5-aminouracil are small, and show a retardation in the replication of nurse-cell chromosomes somewhat similar to that produced in the mutants fs2.1 and sn^{36a}. Chambers are produced as a result, which contain a nonhomogeneous assemblage of nurse-cell nuclei (some resemble stage 3, others stage 4, and others stages 6-10). Numerous Feulgen-positive granules are present in the cytoplasm of oögonia and early nurse cells. Fusions of adjacent chambers occur commonly. Ovaries from flies fed for 10 days show chambers which contain reduced numbers of nurse cells; perhaps the result of nuclear fusions. In these chambers are found two or three giant nuclei, which contain banded chromosomes. A chamber from a fly fed 14 days on 5-aminouracil contained a single nucleus 70 micra in diameter (2.5 X the maximum volume normally observed). Most ovaries from the 14-day series, however, were completely pycnotic.

Further information necessitates modification of the staging of oögenesis of D. melanogaster as given in Growth 20: 121-157. Stage 10 is defined there as the stage at which the oöcyte makes up about half the total volume of the chamber. Stage 10 should be divided into stage 10A, which is common, and stage 10B, which is rare. The dimensions given for stage 10 ($\sim$140 x 360 µ) in the Growth article are for stage 10A. The dimensions for stage 10B are $\sim$190 x 460 µ. Thus the stage-10B chamber is almost the size of a mature primary ovarian oöcyte. In both 10A and 10B the oöcyte and the nurse chamber have equal volumes. Because of shrinkage, the dimensions in Feulgen-stained whole mounts will be 70%-80% the values given above. Feulgen-stained stage-10B nurse nuclei are much paler than those in stage-10A chambers. Thus during stage 10B the 16-cell cyst reaches its maximum volume, and the concentration of DNA in the nurse nuclei falls below that of stage-10A oöcytes. During stage 11 the oöcyte grows at the expense of the nurse cells and eventually reaches its maximum size.

King, R. C., and J. H. Sang. Modification of ovogenesis in D. melanogaster. The Ore-S wild strain studied has about 14 ovarioles per ovary. Freshly hatched flies contain no oöcytes more advanced than stage 7. By one day, all ovarioles contain an oöcyte in active vitellogenesis (stages 8-11) and half the ovari-

oles contain a mature egg (stage 14). By the third day, all ovarioles contain a stage 14 and a stage 8-11; by seven days, all ovarioles have two stage 14's and a third of them have also an oöcyte in active vitellogenesis. If flies are fed on a protein-free diet, active vitellogenesis declines, and after a week no stages 8-11 are found. When such flies are then placed on live yeast, vitellogenesis restarts immediately and over half the ovarioles contain stages 8-11 within 24 hours of the yeast meal. By three days the females are laying normally (30 eggs/day), and they are still laying at this rate two weeks later when each ovariole has an oöcyte in active vitellogenesis. The first dozen or so eggs laid (which were formed during the period of protein deprivation) have a high mortality, but thereafter hatchability is 90% (provided fresh males are supplied).

When the females were placed on yeast their primary oöcytes averaged about 6 per ovariole, and during the subsequent two weeks each fly laid about 340 eggs; so that each ovariole must have produced at least 12 oöcytes, of which half must have originated from oögonia. It follows that keeping a fly on a protein-free diet for a week does not prevent oögonia from forming oöcytes, or oöcytes from synthesizing yolk once protein is again supplied. Protein deprivation for a fortnight does appear to lead to degeneration of germaria and early oöcytes.

When flies are fed on sugar-agar for a week, no stage 8-11 oöcytes remain in the ovary. Yeast feeding for a day then produces large numbers of oöcytes in active vitellogenesis (in line with the above). When such females are irradiated, at least half the ovarioles contain an oöcyte whose nucleus lies in yolky oöplasm, which is increasing in volume. It would be of interest to contrast the mutation rate of such oöcytes with that of normal stage 7's (where the nucleus sits in a yolk-free cytoplasm) and of stage 14 oöcytes, in which the compact genetic material (which is not surrounded by a nuclear membrane) lies in the completely grown yolky oöplasm.

Depriving adults of dietary Mg lowers the frequency of oöcytes in active vitellogenesis, but it does so more slowly than protein elimination, which suggests that the protein store runs out before the reserves of Mg salts. In this case, however, replication of Feulgen-positive material is retarded in the nurse cells. Subsequently, all chambers more advanced than stage 6 degenerate, the nurse cells going before the follicle cells. In spite of this, chambers continue to be produced by the germarium; so that an ovariole may eventually contain as many as eleven oöcytes, of which the posterior six will be degenerating stage 6 and 7 oöcytes and the anterior five will be normal stage 1-6 chambers. So Mg appears to be essential for yolk synthesis, nucleic acid replication, and the transformation of oöcytes from stage 6 to stage 7. Since chamber proliferation fails to occur in the protein-deficient ovary, it appears that protein is required for this process.

Oregon-S, Crianlarach-6, and hybrid females were compared with respect to their ability to synthesize yolk from larval reserves when reared on an inadequate adult diet. The flies were fed on an aseptic 15% starch--0.5% fructose diet during days 0-7. During this period all strains synthesized 8-11 eggs. However, the C-6 and hybrid females laid most of the eggs synthesized at once; whereas the Ore-S females stored the majority of synthesized eggs in their ovarioles. Ovaries from 83 Ore-S females were examined. This corresponds to an ovariole population of 2300. About one-third of the ovarioles contained stage 14 oöcytes. One contained an active first-instar larva. When adults from the three strains were fed on a Mg-free diet, the ovaries of the hybrid were the first to show abnormalities. Thus genotype

markedly influences the effect of dietary deficiencies upon oögenesis.

(This study is part of a collaborative program carried out during the tenure by R. C. King of a National Science Foundation Postdoctoral Fellowship while on leave from Northwestern University.)

King, R. C., and J. H. Sang. Additional description of ap^4.

Adult wild-type females have been fed dead yeast containing various pH indicators (bromophenol blue, bromocresol purple, bromothymol blue, lacmoid), and their digestive tracts have been examined to determine the resulting colors of the material in the lumens. Most of the ventriculus has a pH of about 6. The mid-ventriculus is generally about pH 5, although it can sometimes go as low as pH 3 or as high as pH 6. The pH of the crop varies between 5 and 6. Adult ap^4/ap^4 females live for only 3-4 days and are active only during the first day or so. During this first day the ventriculus of such females is generally pH 5 throughout its entire length. $ap^4/+$ females are normal in this respect. The fore-ventriculus of most ap^4/ap^4 females over a day old is swollen, since it is packed with a transparent material which appears to be disorganized peritrophic membrane. Their cardia appear normal in Feulgen-stained whole mounts. The situation here thus seems similar to that described by Rizki (J.E.Z. 131: 211) for l(1)48j.

Normally, females accumulate sufficient reserves during larval feeding to allow the synthesis of more than half a dozen eggs, even in the absence of an adequate adult diet; but ap^4/ap^4 females fail to elaborate yolk under these conditions, even though they contain large amounts of adipose tissue. No oöcytes more advanced than stage 7 were found in 79 ovaries from 1-2-day-old females: one stage 10 and thirteen stage 14 oöcytes were found in a total of forty 3-day-old ovaries. All other oöcytes were in preyolk stages. No fully formed eggs are ever laid, since ap^4/ap^4 females are completely motionless and their only obvious movement is that of their pulsating hearts and ovaries. The testis of ap^4 males appears normal and contains mature sperm, although the adults are likewise inert. The failure of yolk formation and the blockage of the fore-ventriculus suggested that ap^4/ap^4 females might suffer from a lowered protein intake. Consequently, larvae from the ap^4/al^2 Cy lt^v sp^2 stock were reared asceptically on Medium C (Sang, J. Exp. Biol. 33: 45) and on the same medium with supplemented casein hydrolysate in place of casein, in the hope that they would use these diets more effectively than yeast. However, the diets produced no change in the morphology of wings, halteres, gut, or ovaries, nor did they affect the ability of adults to produce eggs when supplied to them.

(This study is part of a collaborative program carried out during the tenure by R. C. King of a National Science Foundation Postdoctoral Fellowship while on leave from Northwestern University.)

Kroman, R. A. The effect of Ag and Hg ions on melanotic tumor incidence.

In order to test the hypothesis that observed variation in incidence of melanotic pseudotumors is due to the proportion of tumors that become pigmented, and hence recognizable, and not due to the number actually formed by cell aggregation, the effects on tumor incidence of both inhibiters and enhancers of melanin are being studied. Four strains, tu^1, tu^g, tu^{49k}, and tu^{wps} are being tested.

Ag, which inhibits melanin formation, was tested as $AgNO_3$ in concentrations of 0.05, 0.1, 0.15 and 0.2%. Ag ions have a similar effect on frequency in the tu^{49k} and tu^{1} strains, with lower concentrations reducing, and higher concentrations increasing the incidence. Statistically, the minimum frequency, which was observed with concentrations of 0.1 and 0.15%, respectively, in the two lines, was highly significantly less than that of the controls, whereas with 0.2% concentration the incidence was significantly greater than in the controls. The tu^{g} strain exhibited an increasing reduction in tumor incidence with increasing concentrations, which became significantly less than the control value at concentrations greater than 0.1%; the incidence in tu^{wps} remained unchanged at all concentrations tested. It is interesting to note that no correlation was observed between the degree of integumental depigmentation and the presence or absence of a tumor in any individual.

Hg was tested as $HgCl_2$, in concentrations of 0.001 and 0.005%, and in equamolar concentrations of the sulfhydryl inhibiter p-chloromercuribenzoic acid. The compounds had similar effects on tumor incidence, although the latter should increase the incidence if sulfhydryl groups inhibit tumor pigmentation. In the tu^{1} strain there was a marked increase in both penetrance and expressivity, and the penetrance became nearly complete at the higher concentration. In the tu^{wps} strain the incidence was significantly reduced at the 0.005% concentration. In the tu^{g} and tu^{49k} strains, where the controls showed the greatest variation, the incidence was found to be correlated with the variation in incidence of the controls: when the control incidence was high, Hg ions decreased it significantly; when low, they increased it significantly. The last observation suggests that Hg influences the tumor frequency indirectly by its effect on other factors, perhaps the microflora of the culture.

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Kuroda, Y. Comparative study of the wing discs of vestigial series in D. melanogaster in tissue culture.

Wing discs from third-instar larvae of vg, vg^{no}, and vg^{np} strains of D. melanogaster were cultured in synthetic medium (see DIS-30, p. 161), to compare their development with that of wing discs of a wild strain (Oregon).

Wing discs of the vg strain, when taken from mature third-instar larvae grown at 25° C, were distinguishable from those of the wild strain by the size of the wing pouch. When the vg wing discs were cultured in synthetic medium for 24 hours, evagination was observed, with extension of the wing pouch as a cone. When wing discs from the wild strain were cultured, the evagination was characterized by extension of the wing pouch as a cylinder. The difference in evagination between the two strains was observed more and more markedly in further cultivation. The wing discs of vg^{no} and vg^{np} showed characters intermediate between those of vg and those of the wild strain (though more resembling the wild strain) when they were taken from third-instar larvae grown at 25° C. Culture of the vg^{no} and vg^{np} wing discs resulted in evagination with extension of the wing pouch as a smaller cylinder, after 24 hours.

When wing discs from vg larvae grown at 31° C were cultured in synthetic medium at 31° C, evagination was characterized by extension of the wing pouch as a cylinder, as in the wild strain. Cultures of wing discs

of vg^{no} and of the wild strain in synthetic medium at 31° C showed no marked differences from discs of these strains cultured at 25° C. When wing discs from vg^{np} larvae grown at 31° C was cultured in synthetic medium at 31° C, they showed the evagination characteristic of vg wing discs, that is, extension of the wing pouch as a cone. These observations are consistent with the response of the characters of adult wings in these strains to high temperature.

Kuroda, Y., and S. Tamura. Resistance to parathion of various organs in D. melanogaster in tissue culture.

Eye discs, wing discs, cephalic complexes, leg discs, haltere discs, and salivary glands of mature third-instar larvae (about 96 hours after hatching) of the insecticide-resistant strains Hikone and WMB and the insecticide-susceptible strains Fukuoka and Canton-S of D. melanogaster were cultured in synthetic media containing 1.0, 5.0, 10.0, 25.0, and 50.0 ppm of parathion.

When 1.0, 5.0, or 10.0 ppm of parathion was added to the culture medium, no marked effect on any of the organs was observed, even after they were cultured for 48 hours. When 25.0 ppm parathion was added to the medium, it was observed after 24 hours' culture that differentiation and growth of eye discs, wing discs, cephalic complexes, and leg discs were more pronouncedly inhibited in Fukuoka and Canton-S cultures than in Hikone and WMB. Culture for 48 hours in this medium produced marked inhibition of differentiation and growth of these organs, from the resistant as well as from the susceptible strains. The haltere discs of all strains were markedly inhibited after culture for 24 hours in medium containing 25.0 ppm parathion. The growth of salivary glands of all the strains was less inhibited by 25.0 ppm parathion in the medium.

When 50.0 ppm parathion was added to the medium, all the organs mentioned above, from both resistant and susceptible strains, were pronouncedly inhibited after culture for 24 hours.

Lewis, H. W., and H. S. Lewis. Interaction of the tyrosinase-activating systems of Canton-S and sable adults.

Comparison of the rates of activation of tyrosinase extracted from young Canton-S and sable flies revealed that the enzyme from Canton flies becomes activated at a faster rate than does the enzyme from sable flies. This difference in rate of activation of tyrosinase in the wild-type and mutant extracts suggested the possibility of the presence of an inhibitor or inhibition of inhibitors in the extracts of one genotype and their absence in the other genotype. To test this possibility, mixing experiments were performed. The kinetics of the experiments showed that the difference in activation rate between the two genotypes is not due to any inhibition. Furthermore, the maximum activity of the mixed extracts was found to be greater than would be expected on an additive basis. This was explained by the fact that the Canton activator system enhances the activation of the sable proenzyme. This enhancement has been observed in mixtures of 1 part Canton to more than 50 parts sable, whereas the reciprocal mixtures have not shown the phenomenon. Mixtures of the activating system of yellow flies with the sable proenzyme do not show the enhancing effect. The major component of the activating system is a protein.

To test whether the in vitro enhancement could also be demonstrated in

a fly, the activation kinetics of Canton/sable heterozygotes was studied. These experiments revealed that the rate of activation of the heterozygote extract is like that of the Canton enzyme extract, whereas the maximum activity attained is like that of the sable enzyme extract.

Lindsley, D. L., and E. H. Grell. The genetic extent of Dp(3;1)0-5.

Dp(3;1)0-5 is an insertion of a section of 3R extending from 88A-C to 92 into the X chromosome near 4F (Lewis, DIS-27). This segment has been inserted dyscentrically, that is, with region 92 proximal to 88A-C. The ability of the duplication to cover the following third-chromosome markers has been checked: cu (50.0), ry (51.0), kar (52.0), red (55.5), jvl (56.7), cv-c (57.9), sbd (58.2), ss (58.5), bx (58.8), sr (62.0), gl (63.1), k (64.0), and e (70.1). Bridges and Brehme report that sr and e are covered by the duplication, but in the present tests the markers red (55.5) through gl (63.1) are covered and the rest are not. Consequently the genetic length of the inserted piece of 3R is between 7.6 and 12.0 units.

Makino, S., E. Momma, and H. Takada. Drosophila survey in Shiretoko Peninsula, Hokkaido.

A preliminary survey was made on Mt. Raus (altitude 1661 m) on Shiretoko Peninsula at the eastern extremity of Hokkaido, in August 1958. The flies are listed in the table.

Species	Female	Male	Total
Leucophenga sp.	0	1	1
Drosophila coracina	0	3	3
D. bifasciata	50	150	200
D. helvetica	2	3	5
D. testacea	30	32	62
D. nigromaculata	31	8	39
D. brachynephros	3	1	4
D. funebris	4	3	7
D. moriwaki	22	24	46
D. lacertosa	6	15	21
D. sp. (robusta gr.)	3	5	8
D. ezoana	14	7	21
D. histrio	1	1	2
Totals	166	253	419

Meyer, Helen U. A case of double fertilization resulting in parasitism of a genetically lethal portion in a mosaic female.

In the course of breeding tests to detect lethals in ultraviolet-treated second chromosomes, an interesting mosaic female was found in one of the cultures, which (as was ascertained later) contained an induced allele of the dumpy series, a Truncate designated dp^{T57g} (this issue of DIS New Mutants section). The mutant is completely lethal in homozygous condition.

The culture giving the mosaic had contained a cross of a single male of composition $dp^{T57g}.../S^2$ CyInL lt^3 cn bw sp by females of composition S Sp cn.../dp^{txI} CyInsI&R Bl lt^3 cn^2 L^4 sp^2. Offspring representing the class $dp^{T57g}.../dp^{txI}$...were absent, with the exception of one longitudinally

divided mosaic female that manifested the otherwise lethal compound on one side. That side had strong vortices, a blistered wing, and also the characters of Bl, L^4, cn^2 eye color and sp wing. The other side of the mosaic had the constitution dp^{txI} Cy Bl lt^3 cn^2 L^4 sp^2/S^2 Cy lt^3 cn bw sp, as indicated by its phenotype. Homozygous Curly flies of the latter type occasionally survive at a low frequency in uncrowded cultures.

This mosaic female must have arisen by double fertilization of an egg containing the dp^{txI} Cy... chromosome, by the two different kinds of sperm. Though she produced no offspring, she lived for five days, the lethal half of the body obviously being supported by the nonlethal half. It is quite possible that, in a complementary way, the homozygous Curly half was helped to live by the half manifesting the dumpy compound, since the causes of death of the two genotypes would be different.

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Meyer, Helen U., and H. J. Muller. Genetic effects of high doses of X-rays in oögonia.

Even if given as many as 6 doses of 4000 r, adult females were found to undergo some recovery of fertility, provided that these doses were spaced at intervals of several days, 4 days being the interval used in these studies. Some of the females thus accumulated as much as 24,000 r. During the intervals between irradiations the females were kept with males to promote egg production and, thereby, regeneration of oögonial tissue. F_1 females derived from eggs laid from 8 to 12 days after the final treatment were tested for lethals in their irradiated X chromosomes. To reduce the chance of testing more than one F_1 female carrying the same irradiated X chromosome, the P_1 females were bred in groups of 5, and not more than 4 females from any one such group were tested. In a case where two lethals were found in offspring from such a group, localization tests were carried out to throw light on whether they were of independent origin. In all cases they were found to be independent. The P_1 females themselves had obtained the X chromosomes to be tested from their fathers ("P_0"), in which generation it had therefore been nonlethal. Tests of crossing-over frequency, carried out on 9 of the sex-linked lethals, gave one case of marked reduction of crossing over, indicative of a gross structural change. The following results were obtained in experiments utilizing different numbers of treatments according to the above-described plan.

Accumulated X-ray dose	Distribution of doses (4-day intervals)	No. lethals in total no. tests	% lethals
12,000 r	4 x 3000 r	13/244	5.3
16,000	4 x 4000	15/175	8.6
24,000	6 x 4000	8/63	12.7
24,000	6 x 4000	11/62	17.7

Thus the rate of production of sex-linked lethals was approximately 1% per 2000 r (or $5 \times 10^{-6}/r$). The results suggest but do not prove that there is a small dose-dependent contingent of lethals that result in a somewhat

higher rate when individual fractions of 4000 r rather than 3000 r are used; on the other hand, the accumulation of more fractions that are so widely separated as these cannot have caused a real rise above linearity. However that may be, the present induced rate is approximately one-sixth that ordinarily obtained from spermatozoa. Moreover, it is in excellent agreement with the rate of $5 \times 10^{-6}/r$ obtained by Oster for oögonia irradiated in third-instar larvae, in experiments using doses of 600 r and 2400 r (Proc. X Intern. Congr. Genet.: 210-211, 1958). It also is in satisfactory agreement with the frequency of autosomal lethals obtained previously by Meyer after X-ray treatment of the embryonic pole cells (Genetics 42: 385, 1957), where 1500 r to 2000 r gave a rate of $2.3 \pm 1.0\%$ lethals in the second chromosome, since this would be equivalent to about 1% in the X chromosome.

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Meyer, Helen U., and H. J. Muller. Preliminary evidence of detrimental mutations originating at a comparatively high rate in untreated females.

Previous work on the frequency of detrimental mutations (invisible, not fully lethal, somewhat impairing viability and/or fertility) has been confined to those arising in spermatozoa as a result of irradiation, with the exception of that reported in a recent paper by Bonnier, in which X chromosomes derived from irradiated females, although containing lethals at about the expected frequency, gave no evidence of having had detrimentals induced in them. In our present experiments a group of X chromosomes which at the start had been co-isogenic were passed down in separate lines of descent through 53 generations, during all of which they were kept in heterozygous condition in females by means of a genetic mechanism that made use of balanced sterility. At the end of this period one X chromosome of each line was tested for lethals, and if not lethal, for detrimentals. The tests indicated a lethal frequency of some 10%-15%. Unfortunately, many of the lines were lost, but 14 nonlethals of different lines were obtained for testing with regard to detrimental mutations. The tests were of a special kind (to be described in a later report, which involved multiplying the given chromosome and then getting it into males that were placed with attached-X females along with other males containing a similarly multiplied control X chromosome that had originally been co-isogenic with the one to be tested but had not had the latter's opportunity to accumulate mutations. Competition between the two groups of males for the same females was allowed to continue for about 12 generations, and the relative numbers of the two types were then determined by special means. This competition involved both the viability and the fertility of the males--in other words, their total productivity. Several sublines of each of the X chromosomes to be tested were bred in parallel in the same way during this competition period, as checks on the repeatability of the effects found. Among 12 X chromosomes already partially tested in this way, two have so far been found to be definitely inferior to the control chromosomes, and two or three others (on which the tests have not yet been completed) probably inferior. Although the numbers are small, these preliminary results already indicate a frequency of spontaneous origination of demonstrable detrimental mutations in females which is higher than that of lethals.

(This work has been supported by a grant to H. J. Muller and associates from the U. S. Public Health Service, RG-5286 (C1).)

Miyoshi, Y. A new strain of D. melanogaster resistant to NaCl.

A strain resistant to a high concentration of NaCl in the culture medium has been found. It is a strain of bw which has been maintained in our laboratory for a long time but is of unknown origin. To test salt resistance, eggs were removed to a culture medium containing NaCl at a concentration of 1.0 M and left to grow to imagoes. This bw strain is far more resistant than the previously reported se strain (DIS-31); the survival rate of the former is about three times that of the latter (bw, 50.2%; se, 16.7%). By selecting from generation to generation on NaCl-containing culture medium, the survival rate was raised to a level of 90% after 10 generations, and remained almost constant in subsequent generations. In some progenies of the selected strain that were transferred to normal culture medium, the resistance to NaCl has remained at the level of the selected strain through many generations.

Morita, T. Purine contents and xanthine dehydrogenase in D. melanogaster.

It is a well-known fact that the rosy^2 (ry^2) eye-color mutant of D. melanogaster does not contain isoxanthopterin, which occurs widely in Drosophila. Another mutant, ry^1, also contains no trace of isoxanthopterin at any developmental stage. On the other hand, it accumulates larger amounts of hypoxanthine and xanthine at pupal and imaginal stages, but no uric acid, which is an end product of nitrogen metabolism in insects. Purine contents, detected by chromatographic methods, at different developmental stages of Oregon-R and ry are shown in the table. It is very interesting that hypoxanthine is accumulated in the head of the adult fly.

An enzyme, prepared from Drosophila pupae, can catalyze the oxidation of 2-amino-4-hydroxypteridine (AHP) to isoxanthopterin, as well as of hypoxanthine and xanthine to uric acid. This enzyme, therefore, has been called xanthine oxidase or pterin oxidase, but it is a true dehydrogenase, because it requires electron acceptors, DPN or methylene blue, for oxidation. As the homogenate can also catalyze the oxidation of DPNH to DPN, xanthine dehydrogenase seems to link the DPNH-oxidase system by the medium of DPN in vivo. The ry strain does not show any xanthine dehydrogenase activity, but it has DPNH-oxidation activity. The same phenomenon is found in such double-recessive mutants homozygous for ry as v; ry, bw; ry, cn; ry, se; ry, and stw^3; ry.

	Oregon-R		ry		
	Hypoxanthine ug/mg	Uric acid ug/mg	Hypoxanthine ug/mg	Xanthine ug/mg	Uric acid ug/mg
3rd-instar larva	0.0	0.06	0.10	0.10	0.0
Early pupa	0.11	0.70	0.85	0.28	0.0
Mid pupa	0.06	0.80	1.55	0.48	0.0
Late pupa	0.0	1.10	1.88	0.52	0.0
Adult: male					
head	0.33	0.0	2.63	0.0	0.0
body	0.03	0.65	0.37	0.19	0.0
female					
head	0.09	0.0	1.38	0.0	0.0
body	0.0	0.30	0.20	0.20	0.0

Muller, H. J. An androgenetic homozygous male.

From a cross of a heterozygous male having one second chromosome containing cn bw and one third chromosome containing ri e to a female having only normal alleles of these genes, one F_1 male arose that was of homozygous cn bw ri e constitution. There were approximately 100 sibs of expected type. The exception arose too early to be an F_2 and there was no chance for a contamination of the kind in question to have occurred. Since this exception afforded the opportunity of quickly obtaining a stock isogenic and nonlethal for all major chromosomes, it was subjected to crosses for this purpose. It bred satisfactorily but through inadvertence some of the desired descendants were lost and only the third chromosome, ri e, was obtained in homozygous condition. The exceptional male was evidently formed by the union of the nuclei of two paternal germ cells, either after fertilization or during some prefertilization mitotic or meiotic stage, and development must have proceeded with this chromosomal equipment even though the female pronucleus did not make its genetic contribution to the zygote.

Muller, H. J. Pseudo crossing over near centromeres of the third chromosomes induced in late oöcytes by X-rays.

Pursuant of the findings of Herskowitz, Muller, Abrahamson, and Schalet concerning induction of exchanges of diverse kinds between heterochromatic regions by X-rays applied to late oöcytes, a study was made of the frequency of recombinants induced by these means between the central regions of the third chromosomes. The heterozygous mothers were provided with one third chromosome containing ri and p^p and the other third chromosome having inserted into it between ri and p^p close to the left of the centromere an insertion containing a portion of the X with the normal allele of cut. This insertion had been found by Hannah in 1947 as a result of irradiation of the ring X^{c2} and designated Dp(sn R 13aH1). In order that this insertion might serve as a marker in the third chromosome, the females were provided with ordinary attached X's homozygous for y ct and f. They were testcrossed to ri p^p males. The daughters were diagnostic for all three markers in the third chromosomes, the sons only for the two outer ones. The mothers were treated with 2500 r at 125 KVP and changed daily to new broods.

The offspring from untreated mothers contained 6 recombinants between ri and p^p among a total of 1127, or 0.53%, among broods of the first six days. Of these recombinants only 1 occurred among the 433 daughters; this was in the left (ri-Dp) region. The treated females gave 604 offspring (not including here cases of detachment of the X's) from eggs laid in the first four days. These included 20 crossovers between ri and p^p, or 3.3%. This frequency is significantly higher than that in the controls, and when the control value is subtracted from it it indicates that a recombination frequency of 2.8% was induced in these chromosomes by the 2500 r. Among the 206 daughters of treated females here included there were 2 crossovers in the ri-Dp region and 8 in the Dp-p^p region (that containing the centromere).

As a further test of the distribution of crossovers in these two regions in control material, untreated females of the same composition as before were crossed to ct ri p^p males, so that both sons and daughters could now be scored for the ct^+ duplication. Among the 1003 offspring, 5, or .5%, were crossovers in the ri-Dp region and 10, or 1%, in the Dp-p^p region. Although the frequencies here are higher than in the previous controls they remain significantly lower in the right or centromere region than found in the

treated material (1% versus 4%, where they could be scored as such).

These results, taken in connection with others, support the conception that the exchanges induced at this stage are confined largely to the heterochromatic regions near the centromere. It is likely that they occur preferentially between homologous chromosomes, because of the latters' propinquity, but that they do not necessarily occur at precisely homologous positions in the two chromosomes.

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Nawa, S., T. Taira, and B. Sakaguchi. Pterine oxidation in D. melanogaster.

Drosophila homogenate is capable of oxidizing both xanthine and 2-amino-4-hydroxypteridine (AHP). It is likely that the pterine dehydrogenase in Drosophila is a dehydrogenase, not an oxidase, that diphosphopyridine nucleotide (DPN) is a more effective hydrogen acceptor than methylene blue (MB), and that the conversion of AHP to isoxanthopterin is carried out by pterine dehydrogenase in the presence of any DPN in vivo. An enzyme preparation was made from pupae of wild-type D. melanogaster. The freshly prepared supernatant produced a considerable amount of isoxanthopterin from AHP in the absence of any external (exogenous) hydrogen acceptor. When dialyzed enzyme or aged supernatant was used, however, no appreciable production of isoxanthopterin was observed without an external acceptor as shown in the table. Activity is expressed in micromoles of isoxanthopterin produced per gram of whole pupae (wet weight) per hour, pH 8.0.

Acceptor	Preparation		
	Fresh	Aged	Dialyzed
Water	0.55	0.08	0.02
MB	0.9	0.58	0.7
DPN	0.9	0.6	0.75
Cytochrome C	-	0.1	0.02

The activity of pterine dehydrogenase in the presence of DPN has been found to be about equal to or rather higher than that of MB. It seems that the DPN naturally present in Drosophila acts as a hydrogen acceptor. Under aerobic conditions, much more isoxanthopterin is produced than is accounted for by the amount of DPN added. This may be because pterine dehydrogenase is linked to the DPNH oxidase system. The pH optimum of DPNH oxidase in Drosophila is in the neighborhood of 6.5. The ratio of the reaction rate with DPN and with MB as an acceptor is very different at various pH levels. This may be due to the participation of DPNH oxidase in the reaction. The action of the enzyme for pterine oxidation in Drosophila is influenced by the concentration of DPN and the rate of DPNH reoxidation. For example, it has been known that the mutant ry lacks pterine dehydrogenase. The ratio of reaction rates with DPN and with MB as acceptors varies considerably in several different mutants. The variation may be due to differences in the rate of DPNH oxidation. Further experiments along this line are in progress.

Ogaki, M. Effect of genetical background on facet number in some eye mutants of D. melanogaster.

Small-eyed mutants such as B, bar-3, ey^2, and L^2 were subjected to a series of crosses designed to render them co-isogenic with a highly inbred strain of the wild Oregon stock. The shapes of the compound eyes of these co-isogenic strains were compared with those of the original mutant stocks. In B, bar-3, and ey^2 stocks the facet number was increased in the co-isogenic conditions, especially in the case of bar-3 and ey^2. On the contrary, the co-isogenic L^2 stock had a decreased facet number, and in almost all individuals eyes are completely lacking. The facet-increasing effect in the co-isogenic bar-3 stock was analyzed, and was found referable to a recessive gene (or genes) located on the second chromosome of the Oregon isogenic stock. This modifier gene is strong enough in homozygous condition to increase the facets to the extent of twice the number found in the original bar-3 eyes.

Ogita, Z. Genetical study concerning a new type of mixed insecticide for D. melanogaster.

The author (1957) found that the cross-resistance pattern of DDT, BHC, parathion, and PU (phenylurea) is negatively correlated with PTU (phenylthiourea). Genetical analyses suggest that the dominant gene at II-64~66 which confers resistance to DDT, BHC, and parathion also confers resistance to PU and abnormal susceptibility to PTU, whereas the dominant gene at III-50± which confers resistance to nicotine sulfate also confers resistance to PTU as well as PU.

Thus, resistance to PTU and PU is due to polygenic system, which simultaneously requires two main factors on the second and third chromosomes. Therefore, all strains of D. melanogaster may be killed by exposure to a mixture of the minimum amount of PTU that will kill DDT-resistant strains and the small amount of DDT that is enough to kill PTU-resistant strains.

These experiments suggest the possibility that a mixture of DDT, BHC, or parathion with a substance negatively correlated to these compounds, such as PTU, would have effective insecticidal action and would not bring about resistance even after continuous use. Further experiments are now in progress from the standpoint of biochemical genetics.

Okubo, S. N-acetylhydroxytyramine glucoside in D. melanogaster.

It was reported previously (DIS-31) that pupae of the mutant claret (ca) contain a specific phenol. This compound is hydrolyzed by the action of beta-glucosidase, yielding glucose and a hydroxyphenolic compound which exhibits an Rf value of 0.8 in paper chromatography with a solvent containing n-butanol, acetic acid, and water (4:1:1). The hydroxyphenolic compound dissolved in N hydrochloric acid was heated in a sealed glass tube for 12 hours at 100° C, and then the hydrolysate was examined by means of column or paper chromatography (Kirshner et al., J.B.C. 226: 207, 1957; Seki, J. Biochem., in press 1958). It was clear from these experiments that hydroxytyramine (dopamine) and acetic acid were liberated from the dihydroxyphenolic compound exhibiting RF 0.8. Reaction with hydroxylamine, paper electrophoresis, and absorption spectrum were also used to determine the chemical structure. From the results of the above experiments, the specific phenol contained in the mutant claret seems to be N-acetylhydroxytyramine glucoside.

A detailed report will be published in a Japanese periodical.

Oster, I. I., and Astrid Cicak. Mortality of irradiated pre-imaginal stages of Drosophila.

Although a considerable amount of work has been carried out on the sensitivity to X-rays of the pre-imaginal somatic cells of D. melanogaster, the possible influence of sex has not been investigated. Although Mavor had already reported a somewhat higher mortality for male imagoes irradiated during the pupal stage in 1927 (J. Exp. Zool. 47: 63-83), and Patterson had detected a slightly higher sensitivity to X-rays for females than males treated during the early stages of development in 1929 (J. Exp. Zool. 53: 327-372), subsequent investigators (H. Fritz-Niggli, Fortschr. Röntgenstr. 76: 218-254, 1952) have failed to take the role of this factor into account in their work.

By the use of marked stocks, which facilitated the separation of the sexes, it was possible to segregate large numbers of male and female first- and third-instar larvae. F_1 larvae from a cross of two unrelated stocks were used to avoid treating individuals homozygous for deleterious genes. In order to rule out the presence of pre-existing lethals in the female, which would result in a higher mortality among the males, larvae for the controls as well as the irradiated series were collected from the same matings. These were irradiated with either 1280 r or 3500 r (135 kV; 20 ma; 1 mm. Al. filtration; 160 r/min.) when their outer surfaces were fairly dry. Unirradiated but similarly handled larvae served as controls. The following results were obtained:

Treatment	Sex	No. of larvae	No. of dead pupae (late)	No. of imagoes hatching	Unaccounted for	% mortality
Controls	♀	500	5	490	5	2.0
	♂	500	20	480	0	4.0
1280 r (1st-instar larvae)	♀	500	120	250	130	50.0
	♂	500	290	80	130	84.0
Controls	♀	500	12	485	3	3.0
	♂	500	10	482	8	3.6
3500 r (3rd-instar larvae)	♀	500	136	304	60	39.2
	♂	500	340	148	12	70.4

Death very rarely occurred during the larval stages, being very frequent among late pupae. The individuals unaccounted for in the table presumably died during the third larval instar, but because they underwent lytic degeneration we could not detect them when the culture vials were scored. All the individuals that reached adulthood after being irradiated as third-instar larvae showed extreme wing abnormalities, lack of many bristles, and marked weakness. It can be seen in the table that third-instar larvae are about three times more radiation resistant than first-instar larvae, and that female larvae of both stages are much more resistant than males.

Thus far, the only known biochemical difference between males and females which might explain this differential sensitivity, namely, that the

latter contain more free methionine (Kaplan, Science 127: 473-474, 1958) probably does not account for the observed variation, because methionine has been shown to be ineffective as a protective substance against X-rays in mice, although it does protect complex polymers from degradation by X-rays.

It will be of interest to determine whether the difference in radiosensitivity between male and female larvae is of genetic origin. This would presumably be due to the fact that males, having only one X chromosome, would suffer the deleterious effects of its loss after breakage by X-rays more often than females, which have two chromosomes.

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Oster, I. I., Elizabeth Ehrlich, and H. J. Muller. Further study of the mutants f^{x} and f^{+ih}.

In order to throw further light on the peculiarities of the mutants f^{x} and f^{+ih}, previously described (Muller, DIS-20: 88, 1946, and DIS-21: 71, 1947; Muller and Oster, DIS-31: 141-144, 1957, the sublocus of f^{x} was investigated by testing this mutant for crossing over with the mutants f^{3} and f^{1}, the subloci of which normally lie in this order, as shown by Green. One crossover, of normal type, was found between f^{3} and f^{x} among 23,712 inspected offspring, and the markers present here showed f^{x} to be to the right of f^{3}. No crossovers between f^{x} and f^{1} were found in a count of 44,934. Thus f^{x} belongs in the group of those forked mutants which Green found to be to the right of f^{3n} and f^{3} and to be closely (perhaps completely) linked with f^{1}.

To test the effect of increasing the dose of the region containing f^{x}, a chromosome of composition f^{x} B was made up and irradiated, and five deleted X's giving the Bar phenotype were obtained therefrom. All these deleted X's were found, when present in females containing attached X chromosomes homozygous for f^{1}, to have a distinctly normalizing effect on the expression of forked. Thus the region containing f^{x} acts as a hypomorph, not an amorph, even though f^{x} itself has been judged to be a deficiency. The resolution of this seeming paradox can be found in the interpretation that only a sublocus is deficient, so that the locus as a whole, in the chromosome having f^{x}, is in effect acting hypomorphically, in comparison with a normal chromosome. It may be concluded, for one thing, that the absence of induced back mutations of f^{x} in the above-cited work of Muller and Oster, where six would have been expected in the given total number in the case of f^{1}, attests not only to the deficiency nature of f^{x} but also to the infrequency with which duplications arise when spermatozoa are irradiated. The latter circumstance had also been inferred from the sensibly equal frequency with which back mutations of f^{1} had been obtained from irradiated rings and rods, respectively.

That f^{x} is a deficiency, even though of only one or some of the subloci of a complex locus, had been inferred not only from its failure to give back mutations, but (prior to that) from its origination in an irradiated chromosome that was found to carry at the same time a more or less complementary duplication, f^{+ih}, located in the proximal heterochromatin. Tests of f^{+ih} carried out by us have shown that unlike su^{w}-f it exerts considerable suppressing action (dominant) on all other forkeds tried, no matter which sublocus they occupy. Included in these tests, which involved obtaining males with the forked mutant in its usual position and the f^{+ih} near the

centromere of the same chromosome, were f^3, f^{3n}, f^1, f^x, f^5, and f^{36a}, the last two being of an extreme type.

The paradox still remains that f^{+ih}, especially when its variegation is reduced by an extra Y, virtually normalizes the phenotype of a male when f^x (or some other forked) is present in its usual position, despite the distance between the two genetic components here involved, whereas f^x (or any other forked) gives a distinctly forked phenotype in females when in compound with any forked of either region, even though in any such trans combination there are two normal alleles present in much closer proximity to one another than in the former type of case. It is further noteworthy that in the combination of a forked of the left-hand sublocus with f^{+ih} the phenotype is normalized despite the fact that the duplicated element seems to have been derived from the right-hand sublocus, so that the normalizing effect seems only additive here rather than complementary, just as it does when a deleted X containing f^x is added to a male genotype containing f^x itself in its usual position.

The interpretation of these relations which seems at present to be most plausible is that f^{+ih} contains somewhat more of the forked$^+$ region than is absent in f^x. For this to be true, the inserted piece would have had to be derived from a different chromatid, or from a different "singlet" of the Watson-Crick double nucleotide chain, than the piece lost in the f^x deletion, but it would have been inserted into the chromatid or singlet that underwent the latter deletion. Thus the originally left-hand break of the recovered insertion would probably have been further to the left than that of the recovered deletion. At the same time, the argument that f^x does consist of a deletion would remain valid. But the evidence would be weakened that the subgenes of forked represent duplications that occurred in past evolution, since it would not yet have been shown that the normal allele of the f^x sublocus, acting by itself, is able to produce an effect of the f^+ kind.

(This work was supported by a grant given under Contract AT(11-1)-195 of the U. S. Atomic Energy Commission.)

Paik, Y. K. Genetic analyses of lethal mutations in Korean populations of D. melanogaster. Samples of natural populations of D. melanogaster were collected from three remote geographical regions: one each from Najoo, Daegoo, and Quilpart Island in September, October, and September, 1957, respectively. The primary purpose in studying them was to secure more information about the dynamics of natural populations with respect to concentrations of lethals, distribution pattern of lethal genes on the chromosome, and selection of the lethal heterozygotes for two lethals against those for a single lethal. The present report includes only part of the results obtained in connection with the first item mentioned. In all, 472 wild second chromosomes from those regions were analyzed by means of Cy/+ x Cy/+ test matings. The results are summarized in the first table. A glance at the table shows that the proportions of lethal and semilethal concentrations in the populations are extremely low, in comparison with results obtained so far by most other workers. Among the 472 chromosomes tested, the incidence of lethals is only 9.32±1.33%, that of semilethals 2.75±0.75%, and the combined incidence 12.07±1.50%. The table indicates, however, that the frequencies of lethals and semilethals are in general uniform among these three populations. Chi-squares for homogeneity were calculated for the three regions studied, as

follows (d.f. equals 2 for each chi-square):

	χ^2	Probability
Lethals	0.938	0.60
Semilethals	6.935	0.031
Lethals and semilethals	4.36	0.12

The frequencies of intra- and interpopulational allelism were determined for these lethals, as summarized in the second table.

Class viabilities (%)	QI-57i chromosomes		DG-57j chromosomes		NJ-57i chromosomes	
	No.	% (SE)	No.	% (SE)	No.	% (SE)
0 - 3.33	11	7.48 ± 2.16	15	9.74 ± 2.38	18	10.53 ± 2.34
3.34- 6.66	--	---	1	0.65 ± 0.65	--	---
6.67- 9.99	--	---	3	1.95 ± 1.11	1	0.58 ± 0.58
10.00-13.33	--	---	4	2.60 ± 1.28	1	0.58 ± 0.58
13.34-16.66	--	---	--	---	3	1.76 ± 1.00
Total (le. + semi-l.)	11	7.48 ± 2.16	23	14.94 ± 2.87	23	13.45 ± 2.61

Samples	No. lethal chromosomes	No. of crosses	Identical crosses	Allelism rate	No. lethal genes	Frequencies of appearance					
						1	2	3	4	5	6
NJ-57i	18	153	12	7.48 ± 2.17	10	5	3	1	1	-	-
DG-57j	15	105	16	15.24 ± 3.51	9	7	1	-	-	-	1
QI-57i	11	55	1	1.82 ± 1.80	10	9	1	-	-	-	-
Chi-square				8.67							
d.f.				2							
Probability				0.013							

Crosses between populations	No. of cross tests	No. of identical crosses	Allelism rate (SE)
QI-57i x NJ-57i	198	0	-
QI-57i x DG-57j*	198	1	0.505 ± 0.504
DG-57j*x NJ-57i	324	0	-
Total	720	1	0.138 ± 0.138

*Three semilethals are included.

The results show rather significant differences in rate of lethal allelism among the populations. Further, they show relatively high incidence of allelism, although this does not seem to be the case in the QI-57i population; with that exception, about half the lethal chromosomes found in each population occurred repeatedly. On the other hand, all possible intercrosses between the lethals from the different populations showed extremely low frequencies of allelism. If all three interpopulational crosses are pooled as a group, the incidence of allelism is only about 0.14%.

These preliminary data suggest that natural populations of D. melanogaster in Korea are not continuously large but consist of smaller breeding units, with annual cycles of numerical size (Paik, 1957), in contrast to most of the other populations of D. melanogaster or related species that have been studied. However, it seems that there are some similarities, in breeding structure and numerical size of natural populations of this species, between Korea and Russia (Dubinin, 1945). The full results of the present work and the theoretical considerations for it will be published soon.

Plaine, Henry L., and Cheng-Mei Fradkin. The high mutating system in the Swedish-b erupt strain of D. melanogaster.

Following the appearance of erupt eyes, caused by a change in the second chromosome, possibly at the suppressor-of-erupt locus, the Swedish-b strain has been observed to yield a large number of spontaneous mutations. It appears that this increase in the mutation rate is limited also to the second chromosome (DIS-29, DIS-31). During the past year 1,430,676 loci have been tested. Twelve recessive loci on the second chromosome have been tested in 120,552 flies: 68,917 males and 51,635 females. In the males only 5 of the 12 loci yielded mutations, and the average rate per locus was 3.3×10^{-5} (or 7.8×10^{-5} for the 5 loci alone). In the females, mutations occurred at 8 of the loci, with an average rate per locus of 8.96×10^{-5} (or 13.1×10^{-5} for the 8 loci alone). For the males it has not been possible, in the present study, to approximate the 4.2×10^{-4} rate previously reported (DIS-31); however, it is apparent that mutation rates vary in the different subcultures of the Swedish-b erupt strain. In the study reported here, males and females were from the same subculture. Therefore, it seems that the female rate is almost 3 times higher than that of the males. If this is true, it is of considerable interest, not so much because it differs from the findings of other workers, but because it adds import to the possible role of the suppressor-erupt gene, at least in this strain. The expression of erupt eyes is more extreme in the females and there is a significant difference between the low frequencies of affected males and the high frequencies of affected females. The suppressor locus, or at least the second chromosome on which it's located, is solely responsible for these differences in the expression of erupt between the sexes; and these differences are even greater when the Swedish-b second chromosome is derived from the female (DIS-31). The correlation, between the sex-differential suppressor of erupt and the female on one hand and the mutating system and the female on the other, is striking. It is still too early, however, to speculate that the suppressor locus is responsible for the increased mutation rate and may act, therefore, as a "mutator"!

From the tested males, four mosaic male progeny were obtained, but two were sterile and the other two produced no mutant offspring. Thus, the germ

line was probably not affected. However, the tested females produced one mosaic male and two mosaic females, and although the male produced no mutant offspring, each of the two females produced both mutant and normal offspring. Hence, the germ line was partially affected. This supports the concept, offered earlier (DSI-31), that the mutational process may be a delayed one. This is substantiated further by the fact that, in the tested males, the mutation rate steadily increased from 1.9×10^{-5} in the first backcross generation to 7.6×10^{-5} in the sixth backcross generation. Lastly, in at least four cases, normal females gave rise to clusters containing from two to seven mutant offspring. This, together with other data, suggests that the mutator is probably more effective in the heterozygous state.

(Supported by a grant from the National Science Foundation.)

Prout, Timothy. A possible difference in genetic variance between wild and laboratory populations.

The following experiment was designed to detect the operation of stabilizing selection in the wild. It was reasoned that if a wild population was being subjected to some form of stabilizing selection (the favoring of heterozygous genotypes or the favoring of intermediate phenotypes) then when a sample of this population was brought into the laboratory and raised under optimal conditions an increase in genetic variance might ensue. The genetic variances of two groups of male D. melanogaster were assessed by means of a progeny test. One group of 50 males constituted a sample taken directly from a citrus grove population. The other group of 50 males constituted a sample from a laboratory population which had been reared in uncrowded culture bottles and which had been derived one generation previously from the same citrus grove.

Each male was mated to two females, and eggs from each female were collected and distributed in two culture bottles. The females used with both groups of males were from the laboratory population. Wing length was determined for ten females and five males emerging from each culture bottle. The analysis of variance of the resulting data produced a hierarchy of mean squares, and the appropriate "F" tests showed a highly significant culture effect within mothers, mother effect within fathers, and a father effect, but no significant differences between the means of the two populations, fathered by wild and laboratory males respectively. From the mean squares, components of variance were extracted. These components, together with certain other statistics, are set out in the accompanying table. Figures in parentheses are the degrees of freedom for the mean squares from which the components were extracted.

From the point of view of the primary objective of the experiment, the variance components found in the "among fathers" row are important. It will be seen that for both male and female progeny the variance component due to genetic differences among wild fathers is smaller than that due to differences among fathers derived from the same wild population but allowed to grow up under laboratory conditions. At the same time there are no differences in means. These results are consistent with the hypothesis stated above. However, it should be added that according to a statistical test suggested by Crump (Biometrics, 1947) this increase in variance component attributable to laboratory fathers in both cases (2.49 vs. 1.49 for female progeny and 2.33 vs. 1.77 for male progeny) is not significant. Nevertheless the data do suggest that there may have been an increase in genetic variance under the

relaxed conditions of laboratory culture. That such an increase should have shown itself at all indicates that further work along these lines may reveal that joint heterozygosity at a few major loci is strongly favored in the wild.

Variance Components

(1 unit variance = 10^{-4} sq mm)

	Female Progeny		Male Progeny	
	Laboratory Fathers	Wild Fathers	Laboratory Fathers	Wild Fathers
Among fathers	2.49 (48)	1.49 (48)	2.33 (48)	1.77 (48)
Among mothers within fathers	1.30 (43)	2.50 (43)	1.11 (43)	.99 (43)
Among cultures within mothers	1.47 (89)	1.45 (82)	1.09 (88)	1.72 (82)
Within cultures	12.62 (1559)	10.04 (1546)	8.18 (698)	9.65 (698)
Total variance	17.88 (1739)	15.48 (1719)	12.71 (877)	14.13 (871)
Mean wing length	1.536 mm	1.540 mm	1.338 mm	1.341 mm

Parent-Offspring Covariances

Intrafather covariance of mother and mean of her offspring	2.52 (34)	2.28 (30)	1.78 (35)	3.60 (30)
Intrafather covariance of father and mean of his offspring	2.85 (44)	-.93 (47)	2.79 (49)	-2.13 (47)

No serious effort was made to relate these components of variance to statistical models of genic action. However, values of h^2 for wing length ranging from 28% to 73% may be obtained depending upon what one wishes to include in h^2. Using the parent-offspring covariances, h^2 ranges around 36%. This might be the best estimate of heritability "in the narrow sense," since it does not include dominance and particularly is not disturbed by linkage (Cockerham, Genetics, 1956). The appropriate combination of father and mother components (excluding components due to wild fathers) yields a value of h^2 around 48%, which includes various fractions of the nonadditive components plus an unknown disturbance due to linkage. (There is an interesting hint in the data that linkage disturbance may be more important in the fathers' contribution than in that of the mothers, which might be expected in Drosophila.)

Finally, it may be mentioned that the negative covariances between wild fathers and the means of their offspring is interpreted to mean that the wing length of a fly picked up in the field allows no prediction as to the wing length of his laboratory offspring. This fact might be borne in mind by those who have surveyed and are currently surveying quantitative characters in wild populations.

Ramel, C. Interchromosomal effects of inversions on the segregation of Y chromosomes in females.

The effect of autosomal inversions on segregation of the Y chromosome in females with attached and nonattached X's was studied. As autosomal inversions, Ins(2L+2R)Cy and In(3IR)DcxF were used. The following crosses were made: y f:=/sc^8 Y x y w sn/sc^8 Y, y v f.=/sc^8 Y x y w sn/sc^8 Y and y^{16}/y^{16}/Y x Canton-S. In the series with attached X's, missegregation of the Y chromosome was indicated by yellow males in the offspring. In the series with nonattached X's, secondary nondisjunction was counted in both male and female offspring. The following results were obtained:

	y f:=/sc^8 Y		y v f.=/sc^8 Y		y^{16}/y^{16}/Y	
Autosomes	n	% exc.	n	% exc.	n	% exc.
+/+ +/+	5022	2.7	2713	0.2	4632	2.6
Cy/+ +/+	4075	5.4	4999	3.8	4589	1.1
+/+ D/+	1796	7.6	3623	1.7	1735	1.9
CY/+ D/+	2055	3.0	2143	2.9	1105	0.5

In agreement with previous findings (Sturtevant, 1944, and others), the rate of secondary nondisjunction is decreased by the introduction of autosomal inversions. These inversions have an opposite effect on the number of exceptions in the attached-X series. These results could be explained either by assuming an increased pairing affinity between the X chromosomes caused by the autosomal inversions, which would tend to leave the Y chromosome unpaired, or by assuming an interchromosomal pairing of the Y chromosome with an autosome, as has been suggested by Oksala (DIS-31, 1957). If the latter is the case, the Y chromosome would presumably cause nondisjunction of autosomes, resulting in dominant lethals.

An attempt was made to find out whether such an increase in dominant lethals was caused by the Y chromosome. Egg mortality was studied, using y v f.= females with and without a Y chromosome and having the same autosomal inversions as in the preceding experiments.

Egg mortality in per cent

Autosomes	With Y (n)	Without Y (n)	Difference
+/+ +/+	30.2 (1822)	32.6 (3250)	2.4
Cy/+ +/+	48.9 (2711)	57.2 (3228)	8.3
+/+ D/+	68.7 (3613)	71.7 (2051)	3.0
Cy/+ D/+	70.2 (4214)	73.2 (2406)	3.0

All the series show a higher mortality without a Y chromosome. Further experiments are planned to reveal the cause. At present, however, the data do not give any evidence of a mortality caused by the combined effect of autosomal inversions and a Y chromosome. The difference in mortality with versus without Y shows no correlation with the corresponding data on the segregation of the Y chromosome in the preceding studies.

Rasmuson, B., and D. Björkman. The fatty acid content in D. melanogaster.

As part of an investigation concerning the lipid constituents of D. melanogaster, the different fatty acids in the neutral fat fraction was studied

by means of gas-liquid chromatography.

The fatty acid fraction from imagoes was methylated and the methyl esters chromatographed. Nine fatty acids were found, and four of these remained after bromination, showing that they were saturated. These were identified as lauric, myristic, palmitic, and stearic acid. Myristic and palmitic acid were present in about equal concentrations; the concentration of lauric acid was lower, and that of stearic acid still lower. The following unsaturated acids were found: oleic, linolic, a C_{16} acid (possibly palmitoleic), a C_{14} acid, and traces of a C_{13} acid. Oleic acid predominated over linolic; the C_{16} acid occurred in a concentration equal to those of palmitic and oleic acid. The amount of the C_{14} acid was considerably smaller.

Repeated experiments with some different inbred strains of D. melanogaster gave completely identical results, both qualitatively and quantitatively. As corn meal is the major fat-containing constituent of the substrate, the fatty acid content of corn meal was also analyzed. Palmitic acid was the only saturated acid found, oleic and linolic the only unsaturated acids. The concentration of the palmitic acid is lower than that of oleic acid, and the ratio of linolic to oleic acid is reversed in comparison with that obtained from D. melanogaster. Determinations of the fatty acids in the complete substrate, including yeast and the products of microbial action, is under progress.

Rendel, J. M. Selection for scutellar bristle number. Selection for high scutellar bristle number in sc flies results in an increase in average scutellar bristle number. This number is far more variable in sc than in + genotypes. As the number increased in sc flies of a selection line segregating for sc and +, no change in the number on + sibs was found until the average number on sc flies was about 3; a few + flies with 5 scutellar bristles then started to appear. As the bristle number of sc flies increased to 4, the variability began to be reduced; and no sc flies with 5 bristles have yet been observed. As a few + flies with 5 bristles began to appear, the variability of the + segregants began to increase, and flies with 6 scutellar bristles soon followed. There is thus a region around 4 bristles in which genetic changes do not show at all readily. A change which will turn a + 5-bristle into a + 6 or a sc 1 into a sc 3 has little or no visible effect on a + 4-bristle of unselected type.

Rizki, M. T. M. Effect of ligation on tumor production in the tu^W strain. In tu^W larvae the caudal fat cells become encapsulated by hemocytes (lamellocytes), and the masses thus formed are melanized in the late third instar. In one series of experiments, tu^W larvae were ligated so that the brain and ring-gland hormone centers (BRH) were excluded from the posterior part of the body; in the control series for these experiments, tu^W larvae were ligated in such a way that these hormone centers were included in the posterior region of the body. Beginning 6 hours after the time of ligature and continuing at various timed intervals for 120 hours after ligation, larvae in both groups were examined for the development of melanotic masses. In addition to melanization of the caudal fat, which is the specific phenotype of the tu^W larvae, small melanotic masses were noted in various parts

of the body of the ligated larvae in both groups. Such masses are rarely encountered in unligated tu^W larvae, and they occurred with equal frequency in both BRH-excluded and BRH-included larvae. Other experimental evidence has shown that these atypical tumors are due to injury effects. Exclusion of BRH from the posterior part of the body decreases the frequency of the typical melanotic masses of caudal fat of tu^W larvae, and this decrease in frequency of typical tumors is correlated with the age of the tu^W larvae at the time of ligature. BRH-excluded larvae ligated at age 60 hours (24° C) show a complete suppression of the typical melanotic pattern, whereas ligation after 85 hours will slow down the time of appearance of melanosis but will not prevent the formation of the typical melanotic masses. The BRH-included larvae developed typical masses with a frequency of 75% (over-all average based on larvae of all ages) as compared to a penetrance of 95% in the unoperated tu^W strain.

In another series of experiments tu^W larvae were ligated at a time when encapsulation of the fat cells presumably had already occurred but no melanosis was evident by external examination of the larvae. Control larvae were fixed at this time of ligation, and histological examination revealed that encapsulation of fat cells had occurred. The groups of BRH-included and BRH-excluded larvae were fixed for histological examination when 75% of the BRH-included larvae had developed melanotic masses. None of the BRH-excluded larvae had melanized masses. In the BRH-excluded larvae, where melanosis had been inhibited, phagocytosis of the encapsulated fat masses was evident. On the other hand, the melanized masses in the BRH-included series had the typical appearance found in tu^W larvae, and no sign of phagocytosis was observed. Details of these experiments will be published elsewhere.

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Rizki, M. T. M. Feulgen whole-mount preparations of imaginal discs.

Feulgen whole-mount preparations of imaginal discs of the larvae of D. melanogaster have been prepared to study various karyotypes. Larvae (60-65 hours larval age, 24° C) were washed clean and opened in Carnoy solution (3 parts of 95% ethyl alcohol to 1 part of glacial acetic acid). The imaginal disc complex was removed while attached to the mouthparts and the brain, and placed in fresh Carnoy solution for a period of 12-24 hours. The Carnoy was replaced with 95% ethyl alcohol by changing the alcohol solution at least three times, and the material was then left in 95% alcohol for 12 hours. It was then hydrated by passing through a graded series of alcohols to distilled water, with approximately five minutes allowed for each step. The buds were transferred to cold 1 N HCl for 1 minute and then transferred to 1 N HCl at 60° C for 13 minutes. They were placed in Feulgen stain prepared according to Stowell (1945, Stain Tech. 20: 45) for 1-2 hours, and then passed through three changes of freshly prepared bleaches (Stowell method), remaining 10 minutes in each bleach. They were washed in distilled water for 5 minutes, dehydrated through a graded series of alcohols, and cleared in xylol. The entire complex was then placed on a slide and covered with a drop of xylol. Gentle tapping of the mouth parts with a needle freed the imaginal discs and the brain. The mouthparts and the brain were removed from the slide, and the imaginal discs were mounted in Permount. Slides prepared this way must be stored flat to avoid movement of the imaginal discs to the edge of the coverslip. Examinations were made under oil immersion with a green filter on the

microscope lamp.

Somatic pairing can be seen nicely in these preparations. Further, the imaginal discs from five heterozygous inversion strains and X^{C2} show anaphase bridges, and these discs have numerous pycnotic nuclei. The anaphase bridges and pycnotic nuclei are not found in the normal Ore-R strain. Apparently, the high frequency of pycnotic nuclei is due to chromosomal aberrations resulting from somatic crossing over within the inversion heterozygotes. Despite the presence of many pycnotic nuclei in the imaginal discs of these strains with inversions, the imago is by no means morphologically asymmetric or aberrant. A considerable degree of autoregulation of imaginal tissue must, therefore, take place. Perhaps data about frequency of pycnotic nuclei and anaphase bridges in imaginal discs of these strains may be helpful in understanding the development of genetic mosaics resulting from somatic crossing over, by demonstrating the degree of autoregulation of the imaginal tissues that must occur in different karyotypes of Drosophila.

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Rizki, M. T. M. Telobiosis of normal and tumorous larvae of D. melanogaster.

It has been suggested by several authors that melanotic tumors can be induced in nontumorous strains of D. melanogaster by injecting hemolymph and cell-free extracts from genetically determined tumorous larvae. In view of these experiments we have attempted telobiosis of third-instar larvae of the Ore-R strain with tu^W larvae by means of a fine glass capillary. The telobiotic pairs were: tu^W + Ore-R; tu^W + tu^W; and Ore-R + Ore-R. Such telobiotic pairs were kept alive as long as four days. During this period the telescopic movement of the joined larvae was sufficient to produce enough pressure to result in exchange of hemolymph between the partners, and the blood cells could be observed moving through the glass capillary with the phase microscope. In all experiments, the tu^W larvae developed typical tumors characteristic of this strain, but the Ore-R larvae remained free of melanotic tumors even when joined with tu^W larvae. The Ore-R larvae generally pupated.

(Work supported by Grant G-3381 from the National Science Foundation and Grant RG-5285 from the Public Health Service.)

Sakai, K., T. Narise, T. Ito, and S. Iyama. Migrating activity in inbred lines derived from two wild populations of D. melanogaster.

Fifteen inbred lines have been derived from each of two wild populations of D. melanogaster, which differed markedly from each other with respect to migrating activity. Migrating activity was investigated at every generation of inbreeding. The method of investigation was to count the number of flies that had migrated from the original tube 48 hours after the establishment of an experimental set of population tubes. Eighty flies were tested at a time, and the test was repeated three times. The inbreeding was continued to the 20th generation. The following facts were found: (1) The migrating activity of the flies did not show any definite tendency to decrease with inbreeding, though there was a statistically significant variation from generation to generation. (2) The difference between the two populations

was not statistically significant, but intrapopulation variation was highly significant. The line means ranged from 25% to 60% among the lines. (3) Interaction between generations and lines was insignificant. These facts suggest that both populations involve genotypes responsible for either high or low migrating activity, and they were separated from each other by inbreeding. Despite our initial expectation, the inbreeding has not brought about any marked decrease in migrating activity.

Sandler, I., and L. Sandler. An additional case of apparent aberrant segregation of an attached-XY chromosome.

An attached-XY chromosome of the constitution y^+, YSX.YL, y^+ (with the X chromosome in inverted sequence), constructed by Lindsley and Eddington, was tested in the following crosses: (1) y/y x y^+, YSX.YL, $y^+/0$; (2) y/y x y^+, YSX.YL, $y^+/FR2$ (= YL, y^+); (3) y/y x y^+, YSX.YL, y^+/Y^n. The y^+, YSX.YL, y^+ chromosomes were all derived from a single male. The results were as follows:

		Reduction
Cross (1):	877 + ♀♀; 1948 y ♂♂; 2 + ♂♂	55%
Cross (2):	539 + ♀♀; 872 + ♂♂; 2 y ♂♂	38%
Cross (3):	416 + ♀♀; 727 y ♂♂; 1 + ♂♂	43%

In each case it can be seen that the attached-XY chromosome was recovered far less frequently than the expected 50 per cent. The amount of this reduction is shown in the righthand column. There is a great deal of culture-to-culture variability in the ratios, which means that the differences between the different crosses may not be meaningful. In fact, the excess reduction in cross (1) may be due simply to meiotic loss of the univalent attached-XY chromosome.

There are two matters of immediate interest. First, there is the possibility that this depression in the recovery of the y^+, YSX.YL, y^+ chromosome is a consequence of a reduced viability of the X-Y-bearing class. As a test of this possibility, females carrying a normal chromosome marked with y and the y^+, YSX.YL, y^+ chromosome were crossed to YSX.YL, y B/0 males. The progeny included 922 y B ♀♀, 670 B ♀♀, 1169 y ♂♂, and 1065 + ♂♂. Although this is perhaps not the most critical type of viability test, the equality of the y and + male classes suggests that no striking viability depression is associated with the y^+, YSX.YL, y^+ chromosome. A second possibility is that the depression is a result of meiotic loss of the type known to occur with other X-Y chromosomes when such chromosomes are univalent. Cross (2) shows that this is not the case because (a) FR2 acts as a homologue for the X-Y chromosome (as evidenced by the lack of y males) but does not eliminate the reduced recovery of y^+, YSX.YL, y^+, and (b) simple loss of this chromosome should yield a large y male class, which has not been observed.

Although the question of zygote mortality (as measured by unhatched eggs) has not yet been explored, it seems reasonable to suppose that this is a case similar to those reported by Lindsley and Sandler (Genetics, in press), in which other attached-XY chromosomes were recovered much less frequently than the expected 50 per cent from males carrying various X-chromosome heterochromatic duplications. This, therefore, appears to be another case of meiotic drive.

Schlager, G. Fluctuations of pupation site in replicated experiments.

For several years the University of Kansas Drosophila Laboratory has been plagued by unexplainable fluctuations in various quantitative characters. Replicates of an experiment would show significant differences between means, even though the medium was made under standard conditions, the eggs were from the same stock bottles, and the flies were reared in constant environment chambers. Statistical control of these fluctuations was only partially successful (Sokal and Hunter, Proc. Xth Congr. Ent., 1958). It was hoped that these fluctuations could be greatly reduced or eliminated by achieving better control over the environment. The remarks below are confined to pupation site of D. melanogaster (see Sokal and Hunter, Science 119, 1955). The unselected laboratory stock (COSU-2) was used in these experiments.

The quantity and/or quality of the microflora present in the cultures was thought to be the primary cause of the fluctuations. Eggs were washed to remove the yeasts, bacteria, and molds adhering to the exochorion in a manner similar to that of J. H. Sang (J. Exp. Biol. 33, 1956) but using 400 ml of .5% solution of filtered hypochlorite and 400 ml of 2% solution of the commercial germicide "Roccal." Washed eggs were transferred to the rearing medium in a sterile chamber previously washed with 95% alcohol and exposed to ultraviolet light for 24 hours. Smears from the surface of the vials were subcultured in a medium closely resembling the Drosophila rearing medium, to check for contamination.

In each experiment a lot of medium was divided into two parts: washed eggs were transferred to one part and unwashed eggs to the other. Even though possible sources of variation were controlled (i.e., pH of medium, water content of medium, temperature and pressure during autoclaving, temperature and humidity during rearing, microclimatic gradients in rearing chamber, differences between containers of ingredients used in preparation of the medium, differences in eggs collected), the fluctuations still persisted when the standard medium containing corn-meal, agar, yeast, Karo syrup, and Brer Rabbit molasses was used. These differences were maintained in both the "washed" and the "unwashed" vials.

When the eggs were transferred to a medium substituting sugar and mineral salts for the Karo syrup and molasses, the fluctuations were no longer significant in the "unwashed" method but were still significant in the "washed" method. Because of these findings a closer look was taken at the original medium, containing Karo and molasses. When eggs were transferred to original-formula medium containing twice the quantity of Karo but no molasses, the fluctuations disappeared. On the other hand, if the medium was made with twice the quantity of molasses and no Karo, the differences were significant ($P < .01$).

We can conclude (1) that the normal quality and quantity of microflora in the rearing cultures do not alter the expression of the character "pupation-site" enough to cause significant differences in replicated experiments; (2) that differences in pupation site between replicate batches appear to be due to chemical changes undergone by the molasses during medium preparation.

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Schnick, S. M. Viability of heterozygotes and homozygotes for l(2)55i.

Lethal l(2)55i was first found in the W-1(Erie) wild stock. It was maintained by random mating in this stock and in experimental populations at higher-than-expected frequencies. (See Burdick and Mukai, DIS-30, p. 108). Further studies of this lethal have been made with the following results.

Frequency of l(2)55i: After 4 years of random mating q = .1175± in the original W-1 wild stock. Gene frequencies based on 5 generations each of four random-mating experimental populations range from q = .0957± to q = .1647±. These four experimental populations range in age from 31 to 61 generations and are maintained by random mating.

Time of lethality: The gene was found to produce its homozygous lethal effect solely during the larval period. The pupae-larvae ratio of the cross +/l x +/l = .7094, that of +/+ x +/+ = .9305, giving a relative difference in pupation of .2377 which is quite near the expected difference of .25 for a lethal which produces its effects in only one stage. There were no significant differences in larva/egg or in adult/pupa ratios.

Factors influencing high heterozygote viability: The fecundity of females heterozygous for l(2)55i as compared to wild type was measured. Two estimates based on egg laying of 175 females were made. The eggs of each female were counted daily for 6 days, giving a total of 1038 egg counts. One estimate of average number of eggs/female/day gave the values +/l = 77.97 and +/+ = 57.62. This gives a relative fecundity value for the lethal heterozygote of 1.354, that of the wild type being 1. Another estimate of fecundity gives a relative value of 1.21 for the lethal heterozygote, that of the wild type being 1. No difference was found between the wild type and the lethal heterozygote, either in relative zygotic viability or in sperm competitive ability. Differences in mating ability between the wild-type and lethal heterozygous male have not yet been tested.

Seiger, Marvin Barr. Inbred stock of D. melanogaster.

Stocks inbred by single brother-sister pair matings (the number succeeding the name of each strain represents the number of inbred generations as of 58k17):

Oregon-R 250 Single pair received from Ives 56a from the 116th generation.

Iv Oregon-R 270 Same as Ore-R 250 but received 57c at the 231st generation.

M Oregon-R 243 Received from Aloha Alava as a single pair of the 201st inbred generation, 57b. Originally received at Berkeley from Ives as a single pair of the 116th generation 53g. Not inbred from generation 163 to 170.

P_1 I Oregon-R 291 Received from Buzzati-Traverso at the 236th generation 56b.

2b Oregon-R-C 221 Obtained from Aloha Alava as a single pair in the 181st generation, 57b. Stock somewhat sterile and has been mass mated on two occasions for several generations.

f Oregon 180 Obtained from Buzzati-Traverso as a single pair 56b in the 123rd generation. Phenotype: forked.

y Oregon 179 Obtained from Buzzati-Traverso as a single pair 56b in the 121st generation. Phenotype: yellow.

Canton S 56 Received as a single pair in the 14th generation from Aloha Alava 57b. Stock made isogenic by Stern (ClB; Cy/Pm; H/Sb) about 15 years ago. Mass matings until 56h. Single pair matings since '56.

Oregon 100 From Amherst stock #2 (DIS-30) in the 100th generation. Mass mated since.

Oregon 200 From Amherst stock #2 (DIS-30) in the 200th generation. Mass mated since.

2b Oregon-R-C 200 From 2b Ore-R-C in the 200th generation. Mass mated since.

Seki, T., and S. Okubo. Dihydroxyphenolic compounds in D. melanogaster. Since the work of Schmalfuss in 1927, dihydroxyphenolic compounds such as protocatechuic acid have been isolated from various insect cuticles. However, the occurrence of such phenols has not yet been reported in D. melanogaster.

Pupae of an Oregon-R stock were homogenized with 80% methanol, and the homogenate was centrifuged. The supernatant was concentrated under reduced pressure. The residue dissolved in water was acidified with hydrochloric acid and extracted with ethyl acetate. The extract was evaporated under reduced pressure and the residue was dissolved in a mixed solvent composed of acetone, methylethylketone, and 0.2 N hydrochloric acid (1:2:9 v/v). One ml of the resulting solution was placed on a column (0.9 x 110 cm) of Amberite IRC-50 resin (H-form), which had been equilibrated with the same solvent. Elution was effected with the same solvent, and the effluent was collected with an automatic fraction collector (20 drops per fraction). After the addition of 0.2 ml of ethylendiamine to each fraction, they were heated at 50° C for 1 hour and measured fluorophotometrically. Six peaks with yellow fluorescence were found, four of which corresponded with the positions occupied by authentic samples--protocatechuic acid, homoprotocatechuic acid, catechol, and homogentisic acid. Judging from the chromatographic properties, other peaks seemed to represent more polar compounds such as dihydroxyphenyllactic acid and dihydroxymandelic acid.

Seto, Frank. Pupal lethals in combination. As part of a more comprehensive study of the developmental effects of a series of pupal lethals (mentioned in DIS-31, p. 160), different lethals were combined two at a time and the phenotypic expression examined. At the present time 29 combinations have been made and others are in the process of being synthesized. The results of preliminary investigations on the times of action of these "double lethal" combinations are summarized in the accompanying table. The numbers listed in the table are explained in the key and refer to the specific stages at which mortality appeared to have occurred. The stage(s) at which cessation of development occurred for the various lethals taken singly are given in the two below the table and those for the lethals in combination are in the body of the table.

The data show that in practically all cases the double-lethal homozygotes do not develop farther than the stage of the earlier-acting lethal,

and in some cases die earlier. Although the table does not indicate the quantitative effects of these lethals in combination, the numerical data (not presented here) further show that (1) in some cases the nonlethal (Cy/l) heterozygotes are reduced in number and the cultures have increased egg mortality, and (2) there is a general decrease in number reaching the pupal stage and an over-all increase in mortality at earlier stages. Studies are not in progress to determine the patterns of damage produced by the combined lethal effects and to compare them with the effects of single lethals.

N42	*											
X3	2	*										
N61	1,2	2,3	*									
N51	-	2,3	-	*								
N4	-	2,3	2,3	3,2	*							
N50	-	-	-	-	-	*						
N32	-	3	3	-	2,3	-	*					
Co7	-	2,3	-	-	2,3	-	-	*				
Co3A	-	3	1-3	3	3	-	-	-	*			
N1A	-	2	2,3	-	3,4	3	5	-	-	*		
N45	-	2	-	-	-	-	4	-	-	-	*	
N55	-	2,3	2,3	-	2,3	3	-	-	-	4,3	2-5	*
	2,3	2,3	3	3	3-5	4	4,5	4,5	4,5	5	5,3	5,6
	N42	X3	N61	N51	N4	N50	N32	Co7	Co3A	N1A	N45	N55

Key: 1-egg larval
2-larval
3-prepupal
4-early pupal
5-late pupal
6-adult

Shima, T. Drosophila collection in the vicinity of Lake Tōya and the suburbs of Iwamizawa City, Hokkaido.

Flies were collected in the vicinity of Lake Tōya in the summer, and in the suburbs of Iwamizawa City, Hokkaido, during the period from May to October, 1958. The flies obtained are as follows: Aulacigaster leucopeza, D. histrioides, D. coracina, D. buscki, D. auraria A, D. auraria B, D. melanogaster, D. bifasciata, D. brachynephros, D. nigromaculata, D. lacertosa, D. sordidula, D. virilis, D. funebris, D. testacea, D. immigrans, and D. kuntzei. A. leucopeza and D. kuntzei are found rather rarely in Hokkaido.

Shiomi, T. Changes of free ninhydrin-positive substances in the development of D. melanogaster.

In the wild Oregon-RS strain, eggs in three stages, larvae in four stages, pupae in two stages, and imagoes were employed as materials. Ninhydrin-positive substances extracted with ethanol, the final concentration being 80%, were analyzed by means of two-dimensional chromatography. The spots identified were of alpha-alanine, beta-alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, histidine, leucine, lysine, proline, serine, threonine, tyrosine, and valine. Of these substances, alpha-alanine, aspartic acid, glutamine, glutamic acid, glycine, histidine, proline, and valine occurred constantly in each stage of development, whereas the others were not always recognized. Besides these, there were several spots of unidentified substances, of which some were supposed from their Rf values to be peptides, gamma-amino butyric acid, and taurine. The pattern of the ninhydrin-positive substances changes with the advance of the developmental process; each developmental stage shows its specific pattern. Quantitative analyses of these substances are still under way.

Sobels, F. H. Lack of mutagenic effectiveness of two organic peroxides in D. melanogaster.

Radiomimetic properties have been reported by Latarjet (1956) for cumene hydroperoxide and di-succinyl-monoperoxide (DSP) in microorganisms. Samples of these peroxides, synthesized at the Institut du Radium in Paris were kindly placed at our disposal by Dr. R. Latarjet. Oregon-K males were injected with different concentrations of these compounds and their offspring were tested for incidence of sex-linked lethals by means of the Basc (Muller-5) technique.

Cumene hydroperoxide gave 2 lethals in 532 chromosomes and no lethals in 451 chromosomes at the two concentrations tested. No lethals were induced in two successive broods by a highly toxic concentration of 0.1% DSP. Injection of 0.08% DSP produced 9% mortality and 24% sterility. In four successive three-day broods, the following frequencies of sex-linked lethals were observed 3/685, 1/664, 0/545, and 0/531.

Since the spontaneous-mutation rate in this stock is 0.2-0.3%, it is clear that under the conditions tested no positive results have been obtained. Similar observations have been made for DSP in Aspergillus by G. A. van Arkel (1958) and by R. F. Kimball (cited by Latarjet, 1956) in Paramecium. Application of high concentrations of DSP to polar caps of Drosophila by L. S. Altenburg (1958) raised the frequency of second-chromosome lethals slightly over that of the controls.

(This investigation was carried out with support of the Health Research Council T.N.O.)

Sobels, F. H. The effect of pretreatment with cyanide on radiosensitivity in nitrogen and oxygen.

Pretreatment with cyanide enhances the radiosensitivity of spermatids after irradiation in air (Sobels, 1955). A possible interpretation of this effect is that cyanide, by depressing oxygen utilization, makes more oxygen available in the irradiated cells. To test this idea, flies were pretreated with HCN in N_2 or O_2 and then irradiated in N_2 or O_2, respectively. Tests for sex-linked lethals were made by the Basc (Muller-5) method (three virgin females per male per brood). With this mating scheme, the most sensitive stages, corresponding to spermatids, are samples in the second brood. The data of two experiments with N_2 are shown in the upper part of the table; the data of two experiments with O_2 are pooled and presented in the bottom part. Considering the data of experiment 1, it is clear that, compared to radiosensitivity in air, the reduction of radiosensitivity after irradiation in N_2 is more pronounced in spermatids (brood b) than in mature sperm (brood a). In the second experiment, which was aimed at a more effective replacement of air present in the cells by nitrogen, the radiosensitivity of spermatids is leveled off entirely to that of mature sperm. The data suggest that the greater radiosensitivity of spermatids compared to mature sperm is due, at least in part, to a greater availability of oxygen in spermatids when irradiated under normal conditions in air. Our findings are in complete agreement with recent observations of Oster (1957, 1958), who sampled spermatids by irradiating 48-hour pupae.

As to the effect of cyanide in a nitrogen atmosphere, it is apparent that in experiment 1 such pretreatment resulted in a significant enhancement ($\chi^2 = 13.15$; $P < 0.001$) of radiosensitivity in spermatids. After more effective replacement of oxygen by nitrogen in experiment 2, the effect of cyanide

Treatment	Broods (days after treatment)					
	a (1-4)		b (4-7)		c (7-10)	
	no. chrom.	% leth.	no. chrom.	% leth.	no. chrom.	% leth.
Experiment 1						
CN in N_2	462	0.4	474	0.2	478	0.0
1800 r in N_2	597	4.0	712	5.1	712	3.1
CN + 1800 r in N_2	600	3.3	709	10.2	460	2.8
1800 r in air	609	5.4	669	11.7	695	2.4
Experiment 2						
CN in N_2	500	0.4	493	0.2	490	0.0
1800 r in N_2	521	4.4	883	4.1	895	2.8
CN + 1800 r in N_2	524	4.0	885	6.6	889	3.2
1800 r in air	517	5.2	861	10.9	881	3.3
Experiments 3 & 4						
CN in O_2	1123	0.3	1209	0.1	1112	0.1
1000 r in O_2	1139	4.4	1556	8.7	1218	5.3
CN + 1000 r in O_2	1144	4.4	1550	9.7	1002	4.4
1000 r in air	1150	2.3	1217	6.2	1173	4.2

was less pronounced. The results indicate that a small amount of oxygen, which is used up by cellular respiration in the absence of cyanide, markedly increases radiosensitivity in the presence of a respiratory inhibitor. Our findings in Drosophila are comparable to those of Kihlman (1958) with Vicia root tips, where a respiratory inhibitor enhanced radiosensitivity in the presence of minute amounts of oxygen, but not in an atmosphere of pure nitrogen. It seems as if the conditions realized in experiment 2 approach those of pretreatment and irradiation in pure nitrogen in Kihlman's experiments.

A comparison of radiosensitivity in mature sperm and spermatids after irradiation in oxygen and air shows that the effect of oxygen is more pronounced in mature sperm than in spermatids. This observation confirms the idea that compared to the spermatids, mature sperm is relatively anoxic (cf. also Oster, cited above). Contrary to the observations on plant material by Lilly and Thoday (1956) and Kihlman (1957), cyanide if applied in an oxygen atmosphere has no radiomimetic effect by itself. Further, it is seen that pretreatment with cyanide in an oxygen atmosphere does not raise radiosensitivity above that observed after irradiation in oxygen only.

From the data presented above one is inclined to conclude that the enhancing effect of pretreatment with cyanide on radiosensitivity of spermatids in air is due to a greater availability of oxygen. Other findings, however, preclude such an interpretation as the only explanation of this phenomenon. There is no correlation between the effect of cyanide and that of oxygen in spermatids and in mature sperm. That is, compared to radiosensitivity in air, cyanide has a more pronounced effect on radiosensitivity in spermatids than oxygen, whereas the reverse is true for mature sperm. Mature sperm, however, is characterized by a lower availability of oxygen, so that if cyanide acted exclusively by raising the oxygen tension an enhancement of radiosensitivity should be expected in mature sperm and not in spermatids. Both this finding and the fact that posttreatment with cyanide enhances the

effect of high-intensity X-rays (Sobels, 1958) could be explained on the assumption that inhibition of catalase by cyanide favors the accumulation of mutagenic peroxides produced by the irradiation. Since oxygen would be essential for the formation of peroxides by irradiation, the fact that cyanide treatment (pre- or post-) only affects spermatids is in keeping with this hypothesis, because it has been shown that more oxygen is available within spermatids than within mature sperm.

(This investigation was carried out with support of the Health Research Council T.N.O.)

Takada, H. An unrecorded species of the robusta group in Hokkaido.

Eight specimens, 3 females and 5 males, of the robusta group were collected on Mt. Raus on Shiretoko Peninsula at the eastern end of Hokkaido by means of banana-yeast traps. Their characteristics are as follows:

Male and female: Body large, dark brown, about 4 mm in length. Antenna dark brown. Arista with about 7 branches including a fork, 2 below it. Palpus with 2 long and several shorter bristles. Orb-2 about 1/3 orb-1, 1/2 orb-3. orb-2 about 1/2 size of vibrissa. Ocellar triangle large and black. Periorbits black. Carina yellowish brown and high. Cheeks dark brown, about 1/3 as broad as the greatest diameter of eye. Mesonotum dark brownish black, with black median longitudinal stripe. Scutellum brownish black. Sterno index about 0.75. Abdominal tergites brownish black, and with a broad blackish band on each tergite. Legs dark brown. Wings slightly fuscous, veins brown, crossveins clear. C index about 3.7; 4V index about 1.6; 4C index about 0.7; 5X index about 1.4. C3 bristles on basal 2/3. Phallic organs and egg guides unrecorded. Resembles D. lacertosa, Okada 1956.

Takada, H. On the ecological characteristics of D. ezoana (virilis group).

D. ezoana shows a definite ecological specialization. The habitat is restricted to cold and rather damp regions along mountain streams and lakes in eastern parts of Hokkaido. Flies were collected at a place abour 100 m high at the foot of Mt. Raus (altitude 1661 m) in the summer of 1958. There were many butter-burs (Petasites japonicus) and smartweeds (Polygonum reynoutria) covering the area. The meteorological conditions were as follows: average humidity, 100%; range of temperature, 16°-21° C; illumination, 500 lux.

Tamura, S. Resistance to parathion during the developmental stages of D. melanogaster.

First-instar (just after hatching), second-instar (about 55 hours after hatching), and mature third-instar (about 96 hours after hatching) larvae of insecticide-resistant strains (Hikone and WMB) and insecticide-susceptible strains (Fukuoka and Canton-S) of D. melanogaster were raised on foods containing parathion in various concentrations, to investigate the effects of parathion on their pupation and emergence.

In first-instar larvae of Fukuoka and Canton-S, pupation rates and emergence rates were decreased to 4% and 1%, respectively, by 0.1 ppm parathion. In first-instar larvae of Hikone and WMB, pupation rates were decreased to 35% and 26%, respectively, by 3.0 ppm parathion, and emergence

rates to 34% and 26%, respectively. In second-instar larvae of Fukuoka and Canton-S, pupation and emergence were completely inhibited by 0.1 ppm parathion. In second-instar larvae of Hikone and WMB, pupation rates were decreased to 27% and 14%, respectively, by 3.0 ppm parathion, and emergence rates to 26% and 14%, respectively. In mature third-instar larvae of Fukuoka and Canton-S, pupation rates were decreased to 21% and 39%, respectively, by 50.0 ppm parathion, and emergence was completely inhibited. In mature third-instar larvae of Hikone and WMB, pupation rates were decreased to 21% and 19% respectively, by 50.0 ppm parathion, and emergence rate to 3% in both strains.

In the case of first- and second-instar larvae, a slight difference was found between the lengths of the larval periods in the resistant and the susceptible strains, but no significant difference was found between the lengths of the pupal periods.

Toyofuku, Y. Salivary-gland chromosomes of D. lacertosa. The salivary-gland chromosomes of D. lacertosa (♀) are characterized by nine arms: two long, three medium-sized, three short, and one dot. The two long arms have been identified as the X chromosomes. No chromosomal polymorphism has been observed in the salivary-gland chromosomes in natural populations of D. lacertosa, so far as material collected in seven different localities of Hokkaido is concerned.

Tsukamoto, M. DDT metabolism in D. melanogaster. It is well known that in DDT-resistant houseflies, DDT is detoxified by dehydrochlorination to an ethylene-type metabolite, DDE. In this laboratory there are several insecticide-resistant strains of Drosophila (see stock list), and all of them are highly resistant to DDT, although some of them were selected for resistance to other insecticides. Metabolism of DDT to DDE was tested in these resistant Drosophila strains, and the presence of DDE was suggested by the Schnechter-Haller test in ether extracts of larvae, pupae, and adults after rearing on DDT-containing media. However, results of paper chromatography of body extract showed that DDT was not dehydrochlorinated to DDE but converted into an unknown metabolite (or metabolites) having a different Rf value than DDT or DDE. This metabolite appears to be more polar than DDT or DDE. When resistant flies were reared on medium containing DDE, no metabolite was detected on the same chromatographic systems; and the evidence suggests that this unknown metabolite is not a derivative of DDE. Preliminary in vitro experiments for formation of DDE from DDT had negative results. The chemical nature of the unknown metabolite is now under investigation.

*Van Valen, Leigh. Interspecific competition between D. melanogaster and D. willistoni. This experiment was performed last year by the students in the beginning genetics lab at Columbia. Fourteen polyethylene cages were maintained long enough to give information--one by each of 12 students, and two by myself. In all cases but one, D. willistoni was rapidly replaced, adult selection apparently being important.

Except as specified below, each cage was started with 150 males and 150 females of each species. Egg samples of 120 to 1100 eggs were taken a week after the cage was started and at 10- to 20-day intervals thereafter; pupae or adults were counted. Eleven cages were kept at 25°, one at 19°, and two

at 15°. In two cages, strips of absorbent paper toweling were put into the food cups to help regulate cup moisture and to provide additional pupation space for D. melanogaster (D. willistoni usually pupates in the food). In ten cages D. melanogaster was weakened by the presence of either sc cv v f or bw; st. D. willistoni was favored in nine cages by initial proportions of 400:200 or 500:100, and in 8 cages by being placed in one day earlier than D. melanogaster. The generation length is the same within 12 hours in bottles at 25°.

The egg samples from the P_1 generation contained in all but one case a considerably higher proportion of D. melanogaster than the starting frequency, in two cases ostensibly 100% and in three others over 98%. The frequency continued to climb rapidly, although two cages reporting 100% D. melanogaster eggs contained 7% and 3% D. willistoni adults at the end of the experiment. There may have been some interference by D. melanogaster in the egg laying of D. willistoni, D. willistoni imagoes also had a greater propensity to become stuck in the food. Each variable other than temperature had a small effect in the direction expected. The single anomalous cage, at 19°, had the following frequencies of D. melanogaster eggs: 99.2% at 5 days, 99.1% at 21 days, and 52% at 36 days. At 48 days mites were found and the cage had to be discontinued. The adult population then consisted of 74% D. melanogaster. The reason for this situation is unknown; the keeper of another cage at 19° dropped the course before taking any samples.

Waddington, C. H. Genetic assimilation of adaptation to saline media.

Four strains (two derived from Oregon wild types, sp^2 bs^2 and al b c sp^2) have been cultivated for about 20 generations on increasing concentrations of NaCl incorporated in normal cornmeal-molasses media. At the end of this period of intense natural selection, a survival rate of about 15%-20% was achieved by the adapted stocks on 7% NaCl. The length and width of the anal glands (reported to be an osmotic regulatory organs) have been measured in the pupae. Typical figures for the ratio anal length x breadth/pupal length are 0.068 for adapted Ore-K(L) grown in 7% salt, 0.054 for the same stock in normal medium, and 0.044 for the unselected Ore-K(L) in normal medium. Thus the (presumably adaptive) increase in size of the anal organ brought about by selection is partly retained when the adapted strain is returned to normal surroundings.

Wolfsberg, Marilyn F. A note on the egg-laying behavior of mated D. busckii.

In the course of a study involving oögenesis and egg-laying behavior in a standard wild type of D. pseudoobscura, some data were collected for some other species of Drosophila as well. The technique of King (Am. Nat. 89: 369, 1955) was employed to study the daily egg production of individual females. Records of 14 D. busckii females (obtained from Dr. P. S. Woods) were maintained for the first 10 days after eclosion. At 25° C, the developmental period from egg to adult in this species is 12 days; mated D. busckii females reach sexual maturity 2 days after eclosion, on the average. Unlike D. pseudoobscura, D. busckii females do not exhibit any egg-laying rhythm. Under our conditions, the average number of eggs per female per day ranged from 32 to 44 (mean = 38.3). Females occasionally laid large numbers of eggs within 24 hours, for example, 101, 105, 114, 159; but these instances were rare and such females reverted to more characteristic egg-laying be-

havior after the initial period of heavy laying. Kahle-fixed, Feulgen-stained whole mounts of the ovaries of some representative females are being studied. Both the number of ovarioles per ovary and the number of oöcytes per ovariole are greater than those found in the more common species of Drosophila (cf. King and Wolfsberg, Growth 21: 281, 1957).

Wolfsberg, Marilyn F. The effect of ovarian growth on P^{32} distribution in adult D. pseudoobscura.

Newly emerged females were dissected after having fed for various lengths of time (3 hours to 14 days) on a standard cornmeal-molasses-agar medium to which had been added tracer amounts of $H_3P^{32}O_4$. The radioactivity of wings, legs, head, thorax, reproductive system, gut, abdominal residue or fluid residue (hemolymph), which was determined by means of an alpha, beta, gamma restricted atmosphere proportional counter, was expressed as percentage of the total incorporated P^{32}. A record was also kept of the stage of the most mature oöcyte present in the ovary, in accordance with the terminology of King and his collaborators (Growth 20: 121, 1956).

Although there is much variation in the relative distribution of P^{32} in the organs of females during the first 24 to 48 hours, the relative distribution after this time is characterized by a steady state (no change in relative percentages), which lasts until the ovary begins to develop. Growth of the ovary from stage 5 at emergence to stage 14 at sexual maturity upsets the steady-state distribution. After the cessation of ovarian growth, however, the relative percentages of P^{32} in each organ once again become constant. Thus, the relative percentage of P^{32} within the ovary may be correlated with the amount of growth that has occurred in this organ, for example, 5%-8% in the immature (stage 7) ovary, 12%-15% in the intermediate (stage 10) ovary, and 20%-30% in the mature (stage 14) ovary.

Recent experiments have involved keeping females on labeled food until the relative percentages of P^{32} in the various organs reach the distribution characteristic of the female with immature gonads. The flies are then removed to unlabeled standard food and are dissected after various intervals. The relative percentage of P^{32} remaining in each organ is then determined. The results show that during the time the flies are fed on unlabeled food the ovary grows from the immature (stage 7) to the mature condition (stage 14). Despite the fact that the fly has been feeding on unlabeled food during this growth period, the ovary has continued to accumulate P^{32}. Moreover, it accumulates P^{32} until the relative percentage of P^{32} in the gonad has reached the same percentage as that found in the mature ovary of females feeding on labeled food. One cannot explain the data by assuming that the ovary loses P^{32} more slowly than any other organ. The tentative assumption is that the ovary accumulates endogenous P^{32} from other parts of the body during its growth. A metabolic pool of P^{32} mediated b y means of the hemolymph has been suggested. Additional experiments are being planned to test these assumptions.

Yamada, Y., and O. Kitagawa. Polygenic mutation, induced by X-rays, in quantitative characters in D. melanogaster.

The CHI technique proposed by Burdick (1954) was used for this study, except that the fourth chromosome was neglected because our preliminary test showed that the fourth chromosome had little if any effect on hair counts. We treated each sample of five males,

taken at random from an isohomozygous line extracted by the CMI rechnique from a long-inbred line, with (A) 0 r, control, (B) 2000 r, and (C) 4000 r of X-rays, and obtained 36, 47, and 30 homozygous lines, respectively. In each line 40 females and 40 males were scored with respect to number of micro-hairs of abdominals and sternopleurals. The pooled scores of females and males were analyzed. The results so far available are given in the table.

The means for the three treatments show good agreement as regards both abdominals and sternopleurals. However, variances among lines for the same treatment were much higher in B and C than in control A. Variance in A could possibly be attributed to spontaneous mutation, to rarely occurring recombination during isogenization, or to chance sampling; but it still serves as a basis for comparison with B and C. Higher variance after those two treatments, therefore, should be ascribed only to polygenic mutation induced by X-rays. Increment in variance per unit dose was calculated by linear regression and amounts to 0.000167 for abdominals, but is negative for sternopleurals, although the variance in B is significantly larger than in A.

Taking the increment in variance due to spontaneous mutation to be 0.00475, which is merely an average of the values reported by Clayton and Robertson (1955) and Paxman (1956) cited by Mather (1956), the tentatively calculated doubling dose for abdominals is 28.39 or approximately 30 rad. To our surprise, the figure falls within the range of doubling doses estimated for major genes in various organisms. The experiment is still in progress.

Treatment	No. of genomes sampled	Abdominals		Sternopleurals	
		Means	Variances	Means	Variances
(A) 0 r	36	31.2903	0.271378	15.1881	0.439861
(B) 2000 r	47	31.1801	0.924382*	15.1165	0.903444**
(C) 4000 r	30	31.1736	0.940674*	14.9538	0.374808

*Significantly different from control at 1% level.
**Significantly different from control at 5% level.

TECHNICAL NOTES

Arnold, Lloyd L. Culturing flies in disposable paper containers. Drosophila may be bred and nurtured in plastic-lined paper containers, dispensing entirely with the odious and expensive labor of washing and sterilizing fly bottles. The containers can be autoclaved, but we have found it unnecessary. When in use, the containers are covered by a window of clear plastic film, which leaves the entire exposed surface of the medium and the chamber above it open to undistorted observation, either directly or with the aid of a dissection microscope, without removal of the cover.

The basic unit is an 8-ounce plastic-lined "Nestyle" container manufactured by the Sealright Company, Fulton, New York. The containers are 44 mm deep and have an inside diameter of 82 mm at the top and 76 mm at the bottom. When ordered in case lots (250) they cost less than 3 1/2 cents apiece. The feature which particularly recommends the "Nestyle" container for this purpose is the fact that the lip is rolled outwards, down and under, to form a resilient cuff about 3 mm thick and 9 mm wide around the top of the container. This cuff is sufficiently resilient to make a firm closure with the cover, even after considerable use.

The containers are supplied with a hood-type cover consisting of a paperboard ring or hoop about 20 mm high, which fits down snugly over the outside of the cuffed lip, and two paperboard discs, which are folded into the top edge of the ring and cover the mouth of the container. When cultures are set up the discs are pressed out of the ring and discarded. The ring is placed upside down and covered by a sheet of "Saran Wrap." The anesthetized flies are placed on the "Saran" and the container is pressed down into the ring. Small holes may be punched in the "Saran" to increase ventilation.

It is most convenient to anesthetize the flies before opening the containers. For this purpose a simple anesthesia machine can be easily made by placing two or three facial tissues saturated with ether in a polyethylene catsup bottle to the nozzle of which has been fitted a hypodermic needle. The only critical item in this apparatus is the needle; needles larger than #20 make holes large enough for flies to crawl through. The needle pierces the wall of an inverted container and ether-laden air is circulated back and forth between chamber and bellows until the flies are immobilized on the cover. When the cultures are crowded or when the weather is hot, it is better to use a piece of nylon stocking to cover the containers.

For our aging studies we use a cage fashioned from a 16-oz "Nestyle" which permits changing the medium without anesthetizing the flies. The bottom of the pint container is cut off so that it fits nicely into an 8-oz container above the medium, and covered with "Saran" or stocking net. The lower container is changed as indicated by inverting the cage and shaking the flies into the large chamber, while the old bottom is removed and a new container put in its place.

Basden, E. B. A Drosophila suction-collecting apparatus. A sucking-tube or aspirator (as figured in Galtsoff et al., 1937, "Culture Methods for Invertebrate Animals," p. 46) connected to a small vacuum filter pump powered by a water tap makes a very satisfactory apparatus for collecting Drosophila from culture bottles or for transferring them to new bottles when there is no need to examine the flies. A suitably sized cork on the aspirator is fitted into the new vial or new bottle, and the flies can be sucked directly into it from the old culture and may be counted at the same time. This method eliminates the undue handling entailed in the three separate actions of banging into the etherizer, counting on a plate, and transferring. Since the flies are not etherized, there is no risk of their becoming stuck in the medium. If the numbers in the culture bottles are too large and the flies too active for collecting conveniently, they can be shaken into a large glass jar and kept down by a light directed towards the base. By picking the flies off the sides an accurate count is obtained.

Forbes, Clifford. Method of egg collection. Petri half-plates with a 2-inch diameter have been used to collect eggs. The plate bottoms contain the agar medium, which has been moistened with yeast suspension. The parent flies are placed in an empty half-pint milk bottle. Then the Petri dish is put on as a lid. The plates are held in place with masking tape, which is wrapped nearly all the way around, leaving space for air. The bottles are inverted when incubated. This technique allows the collection of as many as 800 eggs from 15 to 20 pairs of parents in a 20-hour period.

Frydenberg, Ove. The Bennett population cages. Bennett (DIS-30, p. 159) described an inexpensive population cage that seems especially useful in experiments where a large number of populations is required. During the last year Bennett cages have been used for different purposes in this laboratory (Institute of Genetics, Copenhagen). Our cages are slightly smaller than Bennett's, and we have found it convenient when working with D. melanogaster populations to change one of the eight food vials every second day, thus leaving any vial 16 days in the cage. Otherwise our set-up is identical with Bennett's.

In sampling the populations, we initially inserted an ordinary 100-mm food vial in the cage and left it for oviposition 24 hours. Comparisons of replicate egg samples obtained in this way from 10 cages showed that the variance between replicates was much higher than the binomial variance. The total homogeneity chi-square was 20.6, with 10 degrees of freedom. This was mainly due to the fact that egg-laying females tended to stay in the rather long vial once they had entered it. A new set of 15 replicate egg samples was then collected from 15 cages, using 50 mm vials with slanted medium; and this considerably reduced the variance between replicates. The total homogeneity chi-square decreased to an insignificant 12.0, with 15 degrees of freedom.

As to the effective population size, one might fear that this at times would be very low in cages that small. We have not yet been able to get any direct measurement of N, but experiments on 16 polymorphic systems in duplicate populations show a high degree of repeatability between duplicates, and thus indicate that random genetic drift is not serious in these cages--at

least not as long as the systems studied are subject to considerable selective drift.

The generation length, defined as the average length in days between two eggs in a line of descent, has been determined by introducing eggs of known age into a marked tester population and recovering the eggs laid by the introduced eggs. The average generation length turned out to be somewhere between 11 and 12 days at 25° C for both populations studied. The distribution of eggs laid shows that the average egg-laying period of the individual females is very short, certainly not more than 48 hours and probably considerably less. The fast turn-over indicated by these preliminary studies may make the Bennett cages favorable tools in selection studies.

Kirschbaum, W. F. A new container for handling vials. Special boxes have been designed which greatly facilitate the handling of culture vials. The latter are placed in the rack inside the box at the time they are washed and they need not be removed again until they are used, since they are dried, filled, and prepared in situ. The boxes are constructed entirely of 1-mm aluminum sheet, painted on the outside. The size of the boxes is such that they are easy to handle and fit two on each shelf of a standard 9-foot refrigerator. Each has a capacity of 80 tubes.

The washed vials are placed in the anodized aluminum rack (which is built into the box) and the box covered with a sliding perforated top. The box is then inverted and placed on the shelf of a drying rack, made especially to hold these boxes. Under each shelf is an inclined aluminum sheet to run off the water and prevent wetting of boxes on the shelf below. Once dry, the tubes may be stored by replacing the perforated top with an entire one. The vials may later be filled with medium without being removed from the rack. (A simple apparatus which fills a whole box of tubes at a time is being perfected and will probably be described in next year's DIS.) The box is then covered with a top made of bronze wire mesh (38 wires per cm) and placed (inclined side down) in a stand, which holds tubes at the proper angle for making the agar slant, for drying. When the agar has hardened, the boxes may be stored in the refrigerator after replacement of the mesh top with an entire one. To prepare the vials for use, cellucotton, yeast, and stoppers may be added without removing the tubes from the rack. On the front of each box is a holder for a card on which may be indicated the contents, date of preparation, etc. If the vials are not to be used immediately, the boxes may be covered again with the entire tops and stored in the refrigerator.

These containers have been in use in our laboratory for the past year and have proved advantageous in the following ways:

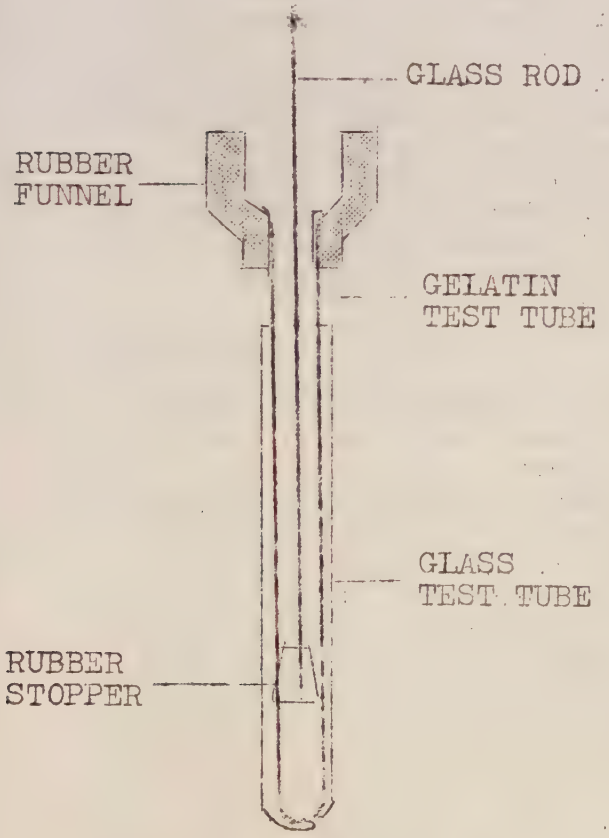
(1) There is less manipulation of vials, and thus a saving of time and personnel.
(2) Washed tubes remain thoroughly clean during storage.
(3) The containers facilitate filling and accelerate preparation of vials.
(4) They diminish the probability of contamination.
(5) Facilitate storage in the refrigerator.
(6) Aid cooling and drying of recently prepared tubes.
(7) Aid the warming to room temperature of prepared vials that have been in the refrigerator, without formation of moisture on or inside the tubes.

Marques, E. K., and C. M. Paiva Maciel. Improved soybean-banana-agar culture medium for Drosophila.

The high protein content of soybean is advantag eous for the improvement of banana-agar media. Experiments have been performed with 5%, 10%, 20%, 30%, 40%, and 50% finely ground soybean flour added to banana-agar medium. The flies hatch earlier and in larger numbers than in the banana-agar medium. Ten pairs of D. melanogaster white mutants were distributed in each tube with approximately 4 cc of food. The tubes were overcrowded. A total of ten control tubes and ten tubes for each soybean-flour supplement were kept at 25° C. On the fifth day the parents were discarded. On the tenth day the flies were counted, and on the fifteenth another count was made. The total yield for 10 tubes on the tenth day was: control, 0; 5%, 0; 10%, 138; 20%, 25; 30%, 40; 40%, 143; 50%, 125. The totals on the fifteenth day were: control, 24; 5%, 11; 10%, 676; 20%, 339; 30%, 500; 40%, 709; 50%, 740.

Maud, G. D., and J. F. Ellis. An apparatus for handling flies without anesthetization.

In our work on modification of low-dose radiation effects by various anesthetics, it became necessary to pair-mate individual flies over a succession of days without the use of ether to immobilize them. An apparatus, pictured in the accompanying diagram, was constructed which permits the operator to eject single unanesthetized flies into mating vials quite rapidly and, with routine care, without loss. The apparatus consists of two nested test tubes, a rubber funnel such as that used with an etherizer, and a glass rod fitted into a small rubber stopper. The outer tube is of glass with a small hole, large enough to permit a single fly through, formed acentrically in the bottom. A corresponding and slightly larger hole is made in the inner gelatin test tube. A one-hole rubber stopper with a glass rod inserted forms a plunger which just fits inside the gelatin tube. The rubber funnel placed on the gelatin tube facilitates shaking the unanesthetized flies into the inner test tube. Twisting the outer tube to align the holes permits the controlled escape of single flies; the plunger helping to concentrate the flies near the openings speed up their escape. By taking advantage of the positive phototactic response of the flies, a light placed behind the recipient mating vials makes it possible to spread the flies even more rapidly and efficiently without anesthetization and without loss.



Mitchell, D. F. A device for obtaining accurate measurements in large samples.

A device constructed by the Drummond Scientific Co. of Philadelphia has proved to be of great value in measuring physical dimensions in large samples of flies. The device consists of a grooved plastic plate, mounted on a stage which moves on ball bearings. This stage is mounted on a second ball-bearing stage, to which is attached a plunger which activates a Federal Pressure Gauge. To obtain the measurements the flies are etherized and positioned in the grooves on the plastic plate; the upper stage is then moved by a screw dial to align one limit of the character with a hair line in the eye piece of a binocular microscope. A second dial then moves the lower stage until the other limit of the character is aligned with the hair piece. Movement of the lower stage activates the gauge, which is calibrated to .01 mm. The dimension is then recorded directly from the gauge, the gauge corrected to zero by turning the lower stage dial, and the next fly aligned with the hair piece by turning the upper stage dial. The device has been used successfully in measuring adult body length, wing dimensions, and puparium dimensions. The accuracy and rapidity of measurement of samples is limited only by the time required to position the flies. Further information concerning the device will be gladly provided upon request.

Pipkin, S. B. Containers for exposing Drosophila larvae to cosmic rays at extreme altitude.

To expose Drosophila larvae to cosmic rays at extreme altitude in balloon flights, light-weight celluloid centrifuge vials 1 1/2 x 6 inches were used. The rounded bottoms of the vials were cut off and shallow stoppers fitted for the cut-off ends. The celluloid vials were sterilized with ultraviolet light; corks and cotton plugs were sterilized in an autoclave. Under an ultraviolet hood, autoclaved corn meal medium was poured into the vials, which were then tilted so as to cause the medium to slant and offer a large surface for egg-laying. After being plugged with sterile cotton, vials were sealed with a paraffin-beeswax mixture at both ends. After exposure, the culture medium containing larvae could easily be taken from a celluloid vial, by opening the cork end with a knife, and shaken into a half-pint milk-bottle of sterile culture medium. There the exposed larvae could complete development without being crowded. Sealed and sterilized celluloid vials with culture medium retained moisture and developed no growth of microorganisms for two weeks before use.

Prout, Timothy. A rapid method for measuring wing length.

Recently many investigators are finding wing length a most useful quantitative character. For those experimental situations where large numbers of individuals are required and where the measured flies may be discarded, the writer suggests the following method. This procedure essentially involves making the measurement after the flies are mounted on microscope slides in Canada balsam. First the flies are overetherized, which almost invariably leaves the wings in a vertical position. Then a microscope slide is streaked with Canada balsam that has been thinned with Xylol. The overetherized flies are then dropped on their sides onto the balsam and allowed to sink so that one wing will be on the surface of the slide itself. If the overetherized flies are first spread on a white card or some other surface they can be transferred to the balsam very rapidly with a moistened pencil or probe. Little or no time is required for positioning the flies after they

are in the balsam. A dozen or more flies can be mounted on each slide, and no cover slip is necessary.

After the balsam has dried (or before) an accurate wing measurement can be made through the back of the slide. The measurement may be made in the usual way with an ocular micrometer; the writer finds the use of a microprojector preferable. By adjusting the height of the microprojector (e.g., Bausch & Lomb Tri-Simplex Microprojector, Cat. No. 42-63-59), the image of the wing can be projected onto a horizontal surface so that with an appropriate ruler the measurement can be made in mm x 100 or some other simple multiple of absolute units. Since the base of the wing is often obscure, the best measurement seems to be between the point of intersection of the third longitudinal vein and the wing margin and the point of intersection of the third longitudinal vein and the anterior crossvein.

As to the permanence of the slides, the writer has taken a series of measurements of the same mounted flies over an interval of five months without detecting any change in this wing dimension. The value of the procedure described is that very large numbers of flies can be collected and mounted at one time and the measurements deferred until later. On one occasion the writer and two assistants were able to collect and mount more than 5000 newly hatched flies from 400 culture bottles in one day.

Shapard, P. B. An inexpensive device for dispensing Drosophila medium.

We have found that an inexpensive cream separator (Montgomery Ward 87FG 4245 M0) serves very well for dispensing Drosophila medium that has been prepared in a pressure cooker.

Slizynski, B. M., and H. Slizynski. Orcein.

During our recent visit in the United States we noticed that some laboratories use "Orcein--extract from lichen" for salivary-gland preparation and do not have good results. The Orcein used in our laboratory here gives very good results; it is Orcein from G. T. Gurr, 136-138 New Kings Road, London, S.W.6.

Sokoloff, A. Types of bristles on the anterior margin of Drosophila wings conspicuous as a result of treatment.

Radius$_1$, the first longitudinal vein, actually possesses two rows of bristles. The dorsal row consists of acute bristles, as indicated by the detail drawing in figure 16, page 408, of Biology of Drosophila, M. Demerec editor. The ventral row consists of blunt bristles shaped somewhat like elongated bullets. In dry-mounted wings the two types of bristles are difficult to discern, even with the highest magnification. They become evident, however, after the following treatment. The flies are killed in 95% alcohol and allowed to remain for two days. A number of flies are transferred to a slide, the alcohol blotted out, and a few drops of paraffin oil added to the mass of flies. The flies are then oriented in a supine position and the wings removed, so that the ventral surface of the wing lies uppermost on the slide. If examined with 40X magnification, a certain proportion of the wings will have an odd appearance. The first longitudinal vein will appear only half as wide as in the "normal" wing, and the anterior margin of the wing will seem darker. After examination with the compound microscope, it is clear that a folding back of the

blunt (ventral) bristles has taken place, leaving the acute dorsal bristles exposed throughout their length. In the few instances in which this process was actually observed, it was seen that usually the bristles proximal to the costal cell begin to fold back, and the other bristles follow suit throughout the length of the vein. In some instances, however, only the bristles located on the middle third or those on the distal part of the vein are affected.

The same reaction can be obtained (1) if the wings are detached from etherized flies and dropped directly into oil (but then a smaller number of wings is affected) or (2) if the flies are mounted in balsam according to the method described by Timothy Prout.

Oddly, populations of Drosophila differ in the incidence of this reaction. The Syosset and Oregon-R population cages maintained by Dr. J. C. King at the Biological Laboratory, Cold Spring Harbor, New York, for several years were repeatedly sampled and treated under the standard conditions described above. Each sample consisted of 50 flies of each sex from each of the population cages. Two points are worthy of mention in these tests: (1) females exhibited the reaction more frequently than males in both population cages; (2) the Syosset population exhibited the character less frequently than the Ore-R population.

*Van Valen, Leigh. Refrigerator boxes as population cages. Polyethylene refrigerator boxes are inexpensive and convenient for maintaining Drosophila populations (Jack Bennett, DIS-30). They are easy to construct, maintain, and clean. The beginning genetics lab at Columbia has since 1957 used 12" x 8" x 4" boxes ($2.49 from Palo Plastics, New York City). Ventilation holes are covered with 60-mesh wire screening; holes for food cups are punched in the bottom, and others are punched in the sides. We use bottle #5901, Will Corp., Rochester, which has a good flange. Food containers are not placed in the side holes because the food tends to run out, even over planed-off corks reaching higher than the food surface.

Because of the pliable nature of the plastic, no more than 10 food cups can safely be placed in the bottom; 8 are used here. This sets a limit on population size disproportionately below that expected by the number of cups. In both D. willistoni-D. melanogaster and D. persimilis-D. pseudoobscura cages, the adult population size ranges between 300 and 800, as compared to 1000-3000 in 16-cup lucite cages. The difference may be due to the lower frequency of introduction of fresh cups, with adverse effects on adults.

Removal of flies from the cages is rather more difficult than with conventional cages, also. Here, and in other cages, it can be facilitated by the following apparatus: A small funnel or the equivalent (e.g., wax in a tube) is put into a cork that has a hole bored through it, and a piece of glass tubing projects from the other end of the cork. A short large-diameter glass tube can be fastened to the large end of the funnel if desired. The cork is placed in a hole in another cage or elsewhere, and the funnel or tube snug to the surface of the original cage in or around a hole. The flies move as readily as normally in the desired direction but virtually never return.

Yamada, Y. A simple technique for identifying sex at the pupal stage.

A very simple but 100%-accurrate method of identifying sex in D. melanogaster at the pupal stage has been used in our laboratory. It should be especially useful to researchers who must work at night or on Sunday morning to secure enough virgin females. The criterion for identification is the sex comb. Through the ventral surface of the pupal case one can easily recognize the sex comb in males, using a binocular microscope at a magnification of 30X, but only after the bristles have become pigmented. Care must be taken (1) not to injure the pupal case when lifting it from the vial, (2) not to allow the pupa to dry out under the microscope (a wet filter paper on the glass plate is recommended).

TEACHING NOTES

Hinton, Claude W. Use of the w^{vc} chromosome in class laboratories.

Several phenomena rarely encountered in undergraduate genetics laboratories can be easily demonstrated in simple crosses involving the unstable w^{vc} ring chromosome (Genetics 40: 951-961), for example, w^{vc} f/y w females by y v f/Y males. As a consequence of elimination of the w^{vc} chromosome, gynandromorphs are frequently encountered among the progeny; those gynandromorphs mosaic in the head may be used to illustrate gene hormones. Exceptional males are also abundant among the offspring; and, although many of these are the result of w^{vc} loss, others are due to primary nondisjunction. This phenomenon is also responsible for exceptional (non-forked) females occurring in the progeny. Position-effect variegation is manifested by the eye pigment of the parental females but not in the F_1 w^{vc} females; the effect of the Y chromosome in suppressing position-effect variegation is demonstrated by the exceptional females' phenotype. The writer will be pleased to supply unstable w^{vc} stocks for this purpose upon request. To insure maintenance of instability, multiple lines of the stock must be carried and selected frequently.

Seto, Frank. Styrofoam population cages for the classroom.

Inexpensive and simply constructed population cages made of styrofoam and glass have been used successfully in this laboratory for the past year. These cages are easily made, relatively durable, usable under most population conditions, and disposable without undue loss in materials and cost. The construction of a typical small cage is as follows. The box frame of the cage is made from a block of styrofoam, about 5" x 6" x 6" in dimension, from which an inner block core is cut out leaving a box with open top and bottom and with walls 3/4"-1" thick. The inner core can be cut out with a thin-blade coping saw or a long sharp cutting blade. The two open ends are

then closed off with 6"-square glass plates, which are held securely in place with masking tape. Eight holes to insert food vials are bored into the walls at an angle to support them with the food ends lowermost. The holes can be made easily with a large-diameter cork borer or by carefully twisting a culture vial into the wall. One of the openings should be plugged with cotton and used for ventilation.

For the best use of the cage, the culture vials should be replaced in rotation, one every other day, so that each vial remains in position for 12-14 days. With continual use, the holes will become larger and support the vials loosely. To correct this situation, masking tape can be bound around the open end of the vial to give a snug fit. A sample of flies can be obtained by removing a vial in which flies have congregated or by collecting newly emerged adults from a 12-day vial. If a total count of the cage population is to be made, the colony can be anesthetized by placing a vial, containing a cotton plug saturated with ether, into the ventilation hole. Then the glass plate is removed by stripping off the masking tape and the flies are swept onto a counting plate. If, after considerable use, the cage becomes too dirty, the flies can be transferred to another cage and the old one discarded.

RESEARCH NOTES*

Anders, G. Fatty acids in D. melanogaster.

The acetone-soluble lipids of D. melanogaster wild-type larvae and flies were saponified and the fatty acids studied by paper chromatography. Three spots with the Rf of palmitic, myristic, and lauric acids could be identified. Three other spots with the same Rf values but from unsaturated acids, probably oleic, linoleic, and an unsaturated acid with the same Rf as lauric acid could be identified. Two lethal factors, lme (2-71-73) and ltr (3-20.7±0.8), were also investigated. Both showed a marked decrease in amount of fatty acids, and lme larvae showed specific damage at the site of the complex spot of myristic and linoleic acid.

Auerbach, C. Effects of heterozygosity for the white region.

A strain of D. melanogaster had been kept heterozygous for the white region by crossing, in each generation for over 10 years, w males to their +/w sisters. Pair matings were made of heterozygous females to wild-type (A) or w (B) brothers; and 30 ♀♀ +/+ from A, 15 ♀♀ +/w from A, 15 ♀♀ +/w from B, 30 ♀♀ w/w from B were tested for fertility by counting the numbers of daughters produced to wild-type males during the first 7 days. In spite of a striking and unexplained difference in the over-all performance of females from A and B, the fertility of homozygotes and heterozygotes within each group was practically identical. Average numbers of daughters per female were: in group A, 112.6 for ♀♀ +/+ and 114.3 for ♀♀ +/w; in group B, 72.0 for ♀♀ +/w and 70.0 for ♀♀ w/w. Thus, in spite of hundreds of generations of enforced heterozygosity of the breeding females, there was no heterotic effect on female fertility.

The larvae of this strain are not subjected to selection for heterozygosity. Competition between homozygous wild-type and heterozygous larvae was tested in the following way. Eight ♀♀ +/w were chosen at random from among the F_1 females of groups A and B, and the genotypes of ten randomly chosen daughters were determined. The data from the two groups were almost identical and have been pooled. Among 157 tested females (3 were lost), there were 96 +/+ and 61 +/w. The difference from a 1:1 ratio is 17.5±6.3. In addition, among the 16 batches of ten females there were 11 with more than +/w, and 5 with equal numbers of the two genotypes. It thus appears that heterozygous larvae were discriminated against in competition with homozygous wild-type ones. The results of Bonnier and Jonsson (DIS-31) suggest that a different result might have been obtained if the genotypes of late-hatching females had been determined; but the experiment had already been completed when this note appeared.

Barigozzi, C. Unexpected appearance of tumors in genotypes with cytoplasm derived from a tumorous stock and chromosomes from a tumorless one.

Combination of cytoplasm from a tumorous stock having 100% (tuB3) tumors with chromosomes derived from a low-incidence stock (Chieti vermilion) was produced in 12 lines independently, each line being bred in several sublines. This combination shows only 2%-3% tumors.

*An asterisk before the title of a note indicates that the author has given unrestricted consent for its citation in publications.

After three generations of mass breeding, one line (6f) proved, quite unexpectedly, to have practically all its second chromosomes rich in tu alleles, producing from 15% to 95% tumors in the isogenic condition. The different second chromosomes behave as nonallelic among themselves, and partially nonallelic with respect to tuB3. Many generations later, a second subline (3c) was found which also had some second chromosomes able to produce tumors in the isogenic condition (up to 43%), but behaved as entirely nonallelic to tuB3. Thus, 3c must be considered an entirely new genotype. It should be noted (1) that 3c has fully normal salivaries, and (2) that the chromosome donor stock fails to show any abnormality as to the production of tumors or as to behavior in Mendelian crosses.

Since errors in the production of the combination cyt tuB3 + chrom. Chieti v should be excluded, the appearance of tu genes in the combination which can also be nonallelic to tuB3 requires an explanation, which escapes the theory of classical genetics. An influence of the cytoplasm upon the chromosomes might explain the phenomenon, but direct evidence of this is still lacking.

Bateman, A. J., and Ann C. Chandley. Mutation spectrum of immature germ cells.

An analysis of the mutation spectrum of immature germ cells in male Drosophila is now nearing completion. In the analysis of induced crossing over in b pr vg heterozygotes, the breakdown of the data into the classes with single and double markers (r_1 and r_2 of Auerbach) has revealed some interesting features. Crossing over should yield r_1 and r_2 in equal frequencies. Point mutations would yield r_1 and r_2 in the ratios of the forward and back mutation rates, which are usually of comparable frequencies. Small deletions would yield r_1 only. In fact, certain samples showed considerable excesses of r_1, which are therefore interpreted as small deletions (they were not analyzed further). Consequently the "crossover" data have yielded two parameters: $r_1 - r_2$ = small deletions and $2r_2$ = crossing over.

By daily sampling, with a mating rate of 2 females per male per day, the period from 2nd to the 13th day after irradiation with 1000 r was analyzed. The period was divisible into four internally homogeneous classes as follows:

	Days			
	2-5	6-7	8	9-13
Total number of chromosomes sampled	17,752	8,533	2,939	15,552
Small deletions, x 10^{-4}	2.8	16.4	-6.8	0
Crossovers, x 10^{-4}	0	9.4	74.9	45.2

Assuming that crossing over is confined to diploid cells, it is inferred that days 2-5 represent irradiated sperm and spermatids, 6-7 spermatocytes, 8 spermatogonia of the last cell generations (hypersensitive to the cell-lethal action of X rays and homologous to "crusty" type-B spermatogonia of mammals), 9-13 early spermatogonial generations. It appears that induced crossing over occurs much less readily in spermatocytes than in spermatogonia, but sperma-

tocytes are particularly susceptible to small deletions, which may arise by illegitimate crossing over.

The general mutation spectrum was obtained only for days 2,5,8, and 11 after irradiation. It is presented below.

	Day				
	2	5	8	11	
Dominant lethals, %	10.8	40.8	60.7*	4.0	
Autosomal recessive lethals, %	5.12	12.19	7.95	3.82	**
Sex-linked recessive lethals, %	2.51	5.32	3.61	1.48	**
Translocations, %	0	3.2	0.8	0***	
Deleted X's, %	0.1	0.3	2.0	0***	
Small deletions, x 10^{-4}	0	2.2#	-6.8#	0	##
Crossing over, x 10^{-4}	0	0	74.9	20.5	##

*Probably spurious, owing to unfertilized eggs.
**Note close parallelism.
***Not recoverable from spermatogonia.
#Not significant.
##See text for further details.

The main objective of this analysis was to search for another mutation type that followed the same sensitivity pattern as the deleted X's, with its continuous rise to a peak at 8 days, a pattern which was confirmed in the present study. Recessive lethals, both autosomal and sex linked, and translocations all show a peak at day 5. If allowance is made for the confounding of dominant lethals with unfertilized eggs, the same may hold for dominant lethals. The only induced change showing any similarity in pattern to deleted X's is autosomal small deletions, though these show a peak at days 6 and 7 (not 8). Small deletions may arise from illegitimate crossing over between paired homologues. Deleted X's could also arise by illegitimate crossing over between the two ends of the single X. This comparison may underlie both the similarities and the differences of the sensitivity patterns of the two phenomena.

It may be noted that there is a very constant ratio (2:1) of autosomal to sex-linked lethals on all sampling days. This appears to contradict the widely accepted view that there is special germinal selection in the male against sex-linked lethals. Two explanations are possible: (a) germinal selection does not assert itself until more than 11 days after irradiation; (b) the low sensitivity of spermatogonia to all types of mutation has been mistaken for germinal selection.

*Baumiller, R. C. and I. H. Herskowitz. X-ray-induced delay in egg hatching due to eucentric mutations in heterozygous condition.

Virgin y females were mass-mated in bottles for two days to equal numbers of + males which had or had not been treated with 3000 r. The parents were then transferred to nylon sacks, each containing a total of about 4000 individuals, in which the females were able to oviposit for two-hour periods upon dishes containing sugar-agar medium seeded with yeast. Most of the eggs were laid during the second hour of each period, since the flies required about one hour to become adjusted after each change of egg-collecting dishes. Each hour, from 18 to 30 hours after the dishes were removed, all hatched larvae were counted and discarded. The temperature throughout the experiment was 25°±1° C.

The table shows the combined results from two such egg-laying periods. Results for hours 18, 19, and 20 are omitted, since the larvae came from eggs which had been fertilized and held by the females for various lengths of time before being laid. Supporting this view is the fact that, except for hour 30, hour 20 had the lowest percentage of individuals hatching.

Of all individuals hatching in the unirradiated control, the peak value of 43.6% was reached 24 hours after the dishes were removed. During each of the hours 25-30 the percentages hatching were higher in the treated series than in the control. The percentage hatching during the 25th hour approached a significant difference ($P=0.08$), and the differences during hours 26, 27, 28, and 29 were individually significant ($P < 0.05$). The difference for hour 30 was not significant.

Since individuals that hatch from eggs comprise a sample which contains few or no gross ploidy changes, it is concluded that after 3000 r of X-rays approximately 17% of individuals were delayed in hatching (some for several hours) owing to euploid, or near-euploid, mutations in heterozygous condition.

(This study was supported by a grant from the U. S. Atomic Energy Commission, Contract AT(11-1)-633.)

Hour	No. Hatching/Total Hatching % Control	% Irradiated	P
21	1.15	2.30	0.10
22	2.75	2.43	0.53
23	17.87	17.03	0.85
24	43.64	26.89	< 0.0001
25	23.83	27.70	0.08
26	5.50	11.35	< 0.0001
27	3.21	6.22	0.006
28	1.72	4.19	0.0045
29	0.23	1.62	0.006
30	0.11	0.27	0.99
Total larvae	873	740	

Brosseau, George E., Jr. The autonomy of bb.

In his original study of somatic crossing over Stern (1936) noted that mosaics for bb were not observed where they were expected. This means either that bb is nonautonomous or that all

the exchanges take place distal to bb. In order to determine whether bb is autonomous or not, two different bb mutants and one bb^1 were crossed to w^{vc}/delta-49 females. In the case of the mutant bb's, gynandromorphs were readily obtained and showed the bb phenotype clearly in the male parts of the flies. The bb^1 failed to yield any gynanders, a result indicating autonomy of the lethal effects. Thus bb is completely autonomous and the likely answer to Stern's results is that somatic exchanges proximal to bb ... seldom or never occur.

Brosseau, George F., Jr. Further evidence that bw^D is a position effect.

Slatis (1955) presented evidence that bw^D is a position effect. However, in his studies he did not assess the effect of the Y chromosome on bw^D. As it is difficult to apply most of the usual criteria for position effect to bw^D, the effect of the Y provides the only critical test for position effect. The following genotypes were produced in order to test the effect of the Y on bw^D: y v/Y; bw^D/bw vs. y v/0; bw^D/bw, and yy/0; bw^D/bw vs. yy/Y; bw^D/bw. In each case the Y chromosome had a small but clearly discernible suppressing effect on the bw^D phenotype. Similar genotypes with bw^D/+ were also examined, with similar results, although the effect was not so easily seen because of the large amount of pigment present. It can be concluded that, with respect to the effect of the Y chromosome, bw^D fits the pattern of a V-type position effect.

Browning, L. S., and Edgar Altenburg. Lack of evidence of a lowering of the rate of ultraviolet-induced lethal mutations by posttreatment with chloramphenicol in Drosophila.

In view of the importance of the discovery by Witkin that chloramphenicol posttreatment reduces the rate of mutations induced in bacteria by ultraviolet, it was considered desirable to test the extent of this effect in other organisms. Accordingly, Drosophila eggs at the polar-cap stage were dechorionated and the polar-cap cells (representing the germ track) were irradiated with a dose of 2537 A ultraviolet light from a germicidal lamp at 180 cm for 3 1/2 minutes (a dose of approximately 180 ergs/mm^2), after which the polar caps were immediately immersed in a 0.1% aqueous solution of chloramphenicol for 20 minutes in the dark, then removed to food medium containing 0.1% chloramphenicol and allowed to develop. Another group of eggs was treated with ultraviolet only, and a third group was exposed for 20 minutes to 0.1% chloramphenicol placed directly in contact with the polar caps, then removed to food containing 0.1% chloramphenicol. All the eggs that developed into fertile males were tested for recessive lethals in the second pair of autosomes by means of Muller's sifter technique. The mutation rates of the three groups were as follows: ultraviolet only, 2.0±0.8%; chloramphenicol only, 0.9±0.4%; ultraviolet plus chloramphenicol, 2.4±0.7%. Although the mutation rate for the 3 1/2 minute ultraviolet treatment is lower than that usually obtained with this dose, it is evident that no significant lowering of the rate was caused by posttreatment of the polar-cap cells with chloramphenicol. Possibly, however, the chloramphenicol was not given sufficient time in which to act. The polar-cap stage lasts for only about a half-hour after the eggs have been prepared for treatment, after which time the polar-cap cells leave their exposed position at one end of the egg and migrate

into the interior of the egg, where they no longer are directly exposed to outside agents.

(This work has been supported by N.I.H. grant C-2393.)

Browning, L. S., and Edgar Altenburg. The non-effect of relatively short interruptions of treatment on the mutation rate induced in Drosophila by ultraviolet.

The germ cells of Drosophila in the polar-cap stage of the developing egg were treated with ultraviolet in order to determine the relative mutagenic effects of intermittent versus continuous ultraviolet treatment. In the series of experiments involving continuous treatment, the cells were treated for 3 1/2 minutes at a distance of 180 cm from the source (a germicidal lamp), the dose being 180 ergs/mm^2. In the intermittent series, the distance of the cells from the lamp and the total dose of ultraviolet were the same, but the treatment was given in 15-second periods with a 15-second interval between periods. The lethal rate in the second chromosome was determined by means of Muller's sifter technique. The rates were as follows: continuous treatment, 4.1±1.0% (39 lethals in 946 tested chromosomes); intermittent, 4.1±1.3% (31 lethals in 755 tested chromosomes). Thus no significant difference was detected between the mutation rates in the two series. However, in view of the fact that photorepair of ultraviolet-treated material is possible as long as a half-hour after ultraviolet treatment, it is possible that the interval between periods of treatment in the intermittent series was not sufficiently long to prevent the effect of a "hit" in one period of treatment from acting synergistically with hits in following periods.

(This work has been supported by N.I.H. grant C-2393.)

Carlson, Elof A., and J. L. Southin. Preliminary pseudoallelic analysis of X-ray-induced mutations at the dumpy locus in D. melanogaster.

A problem of extreme interest in radiation genetics is the extent of minute structural damage to genetic material by ionizing radiation. Two general approaches may be used to detect such rearrangements: first, a phenotypic analysis, testing the induced mutant with genes adjacent to it for evidence of deficiency or position effect; second, a recombinational analysis, testing the mutant with its neighbouring genes for the presence or absence of crossing over. The recombinational approach has not been widely used for pseudoallelic regions whose mutant sites have been sufficiently mapped. For this reason a series of X-ray-induced dumpy mutations obtained at doses of 4000 r and 1000 r, administered to mature spermatozoa, were examined for gross and minute rearrangements in the region bounded by echinoid (ed--2-11.0) and clot (cl--2-16.5). This screening (see Carlson, GENETICS 44: 347-373) left a number of mutants that could be considered point mutations. For a further, more defined, level of analysis these mutants are being subjected to pseudoallelic examination. Two series of mutants, showing lv and olv phenotypes (where o represents oblique wings; l, lethality; and v, thoracic vortices), are particularly valuable for this study. In the tentative map sequence, l--ol--olv--o--lv--ov--v, the lv mutants could represent changes that occur between o and ov, since these members express the o effect, which lv does not. Alternatively, one might interpret some lv phenotypes as recessive lethal rearrangements at the v site. The olv class could

easily represent a mixed group of partial or complete losses of genetic material in the dumpy region as well as gene mutations at the olv site. Both lv and olv types were tested with ed.v^2 cl. Of five members of the lv class so far examined, all show recombination to the left of v^2, with frequencies generally similar to that of the spontaneous mutant lv^1. Of five members similarly examined in the olv series, all recombine to the left of v^2, but show a greater variation in frequency of recombination than do the members of the lv series. These results are presented in the accompanying table. Although they only represent about half the mutants available for this type of analysis, the trend lends itself to interpretation. First, even at high doses of radiation, a significant portion of mutations at the dumpy region represents changes which reflect lesions at a considerably finer level than loss or rearrangement of the entire gene itself. Some of these, such as lv, are almost entirely gene mutations occupying less than 20% of the dumpy region (namely, the region between the o and ov sites). Others, such as olv, reflect changes of various sizes falling into two categories: minute rearrangements sufficient to disturb crossing over in the echinoid-clot region, and lesions generally smaller than the dumpy region itself. That these olv categories are distinct is suggested by the rarity, so far, of mutants capable of crossing over with ed and cl but unable to recombine with v^2.

Mutant	Origin*	Confirmed ed + recombinants	Total count	Frequency**
olv^{x1}	HS	2	16,947	0.024
olv^{x2}	LS	4	7,100	0.112
olv^{x3}	HI	1	14,230	0.014
olv^{x5}	LI	5	28,270	0.035
olv^{bm}	HI	7	16,795	0.083
olv^{1}	spont.	6	7,875	0.152
lv^{x1}	LI	1	1,795	0.110
lv^{x3}	HS	2	3,835	0.104
lv^{x4}	HS	1	7,675	0.026
lv^{x5}	HI	3	4,597	0.131
lv^{x18}	HS	6	12,428	0.097
lv^{1}	spont.	14	26,280	0.107

*H = 4000 r, L = 1000 r, S = spermatazoa in males, I = spermatazoa in inseminated females.

**Frequency doubled since double-mutant class is not detectable in these crosses.

(This work was partially supported by grants to Dr. H. J. Muller and associates from the U. S. Atomic Energy Commission, Contract AT(11-1)-195, and to the senior author from the National Research Council of Canada, grant number A776.)

Chovnick, A. The use of c3g in attached-X experiments. Methods for the study of crossing over within sex-linked complex loci utilizing attached-X females have been described by Lewis (PNAS 38: 1952). The chief advantage of this method is that one

recovers two of the four meiotic products, and it is possible to recover reciprocal crossover products. The chief disadvantage is the restriction on sample size enforced by the method. Complete counts must be made of all classes of offspring from single female matings in order to follow the relative position of heterozygous markers. This practice is self-defeating when one is analyzing very small regions in which large samples are important. By utilization of the third-chromosome recessive suppressor of crossing over, c3g, it is possible to avoid the difficulty. Attached-X heterozygous females, homozygous for c3g, are maintained in stock by crossing to c3g homozygous males. Crossing over in such females is suppressed, and the heterozygous markers remain in known relative position. An outcross of such females to males carrying autosomal inversions provides a population of attached-X females with heterozygous markers in known position, possessing autosomal inversions to stimulate crossing over, and heterozygous for c3g. Mass matings involving such females permit large sampling and the half-tetrad analysis feature is preserved.

This method was adopted for use in analysis of the garnet locus in D. melanogaster. In addition, it was useful in providing heterozygous attached-X females with known marker positions for genetics class experiments demonstrating four-strand crossing over and tetrad analysis.

Coomes, Roger K., and Jack Bennett. Apparent inverse lethality in DDT-resistance testing.

In testing resistant strains of D. melanogaster in the World Health Organization Mosquito Test Kits it was noted that with a long exposure time (18 hours) greater lethality occurred in the control vials than in the lowest DDT concentration. The effect was absolute: no DDT vial of the lowest concentration (0.25%) had higher lethality than its paired control vial. This effect did not occur at shorter exposures (1 to 8 hours), but these exposures were insufficient to gauge the resistance of the stocks tested. It is hypothesized that a component of the resistance is based on a behavior change that causes the flies to leave a surface that has DDT on it. Both DDT and control vials were partially lined with paper saturated with mineral oil (as a vehicle for the DDT). The flies are supposed to have stayed away from the DDT and oil combination, but to have remained on the oil when DDT was absent, picking up enough oil to kill them. Nonresistant strains showed no such effect.

*Counce, S. J. Micropyle structure in the eggs of several Drosophila species.

During a comparative study of the embryology of nine species of Drosophila (D. melanogaster, D. willistoni--subgenus Sophophora; D. virilis, D. americana, D. replata, D. hydei, D. gibberosa, D. funebris--subgenus Drosophila; and D. buschii--subgenus Dorsilopha) variations were noted in several egg and embryonic characteristics. Species variation in the morphology of the polar granules and the behavior of the pole cells has been reported elsewhere (Anat. Rec. 134: 546). The present report deals with variations in the structure of the chorionic micropyle as observed in sectioned material.

Shape of micropyle:
D. melanogaster, D. willistoni: nipple-shaped.
D. virilis, D. americana, D. funebris: short, broad, with spur at base of ventral side. D. funebris--tip slightly broadened.

D. repleta, D. hydei: Micropyle elongate and slender, with slightly flattened tip; shape reminiscent of an old-fashioned wooden clothes pin.
D. gibberosa: length and shape comparable to D. melanogaster except for ring of chitinous hooklets surrounding rather heavy tip.
D. busckii: length and shape like that of D. melanogaster except for few hooklets at tip and spur at ventral base.
In closely related species (e.g., D. repleta and D. hydei) the micropylar structures are very similar.

Micropyle cap: In some species, the anterior tip of the micropyle is covered with a cap of noncellular material, which stains yellowish-gray with iron hematoxylin, and through which small canals apparently extend to the micropylar opening. Caps are apparent on all eggs of D. melanogaster, D. willistoni, D. virilis, D. americana, and D. busckii and in approximately half the eggs of D. gibberosa. No cap has been observed on the micropyle in eggs of D. funebris, D. repleta, or D. hydei. The chemical nature, origin (ovarian or oviducal, pre- or post-insemination), and function of this structure are not yet known.

*Counce, S. J. Spermatozoa in Drosophila eggs. Polyspermy is the general rule in Drosophila. A comparative study of the embryology of nine Drosophila species (listed in report above), however, has shown there is considerable specific difference in the degree of polyspermy. Five to six spermatozoa (and rarely more than ten) are typically found in the eggs of D. melanogaster and D. busckii; approximately the same degree of polyspermy is to be found in D. willistoni, D. repleta, and D. gibberosa. Approximately three to four times this many sperms occur in eggs of D. funebris and D. hydei, and an even higher degree of polyspermy exists in D. americana and D. virilis. In these two latter species, 50-100 sperms are regularly found; moreover they are all arranged in one or two loosely coiled bundles, whereas in other species so far examined the supernumerary sperms occur singly although they are usually restricted to the anterior third of the egg (but see below).

Supernumerary sperms apparently do not disturb cleavage or cell divisions, contrary to the report of Huettner (Zeit. Zellforsch. mikroskop. Anat. 19). Sperm tails may remain visible in the embryo for several hours. In D. americana and D. virilis the sperm bundles are eventually shunted into the primitive gut and are finally included along with the yolk in the lumen of the midgut. In other species, supernumerary sperms are often expelled into the stomodaeal cavity. Supernumerary sperms have also been observed extending from the micropyle to the superficial surface of the embryo as late as the cellular blastoderm stage. Sperm heads are visible only during the earliest stages of development.

In D. funebris and D. hydei spermatozoa may penetrate 2/3 to 3/4 the length of the egg. In D. hydei, this may be related to the fact that the maturation divisions of the egg nucleus occur in a dorsal region about half-way back in the egg instead of in the more anterior region under the chorionic filaments characteristic of other species. In D. funebris, however, the polar nuclei are formed in the region of the filaments in spite of the fact that sperms may also penetrate to a greater depth.

Large numbers of sperms adhering to the entire surface of the chorion

have been observed on eggs of all species so far studied. This suggests that the insemination mechanism results in a considerable wastage of spermatozoa. A fertilization cone extending into the micropyle region during the maturation divisions and synagamy has also been observed in the nine species studied.

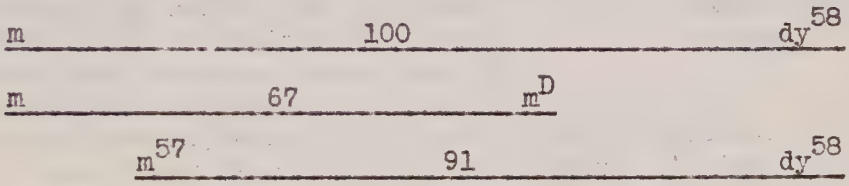
Di Pasquale, A. A new character of D. melanogaster, "brown spots." In different stocks of D. melanogaster individuals of tumorous genotype have been found which show--in the females only--irregular brown spots in the hypodermal cells. Tumors appear in both sexes. The character is controlled from the second chromosome (like the tumors) and appears in isogenic combinations. It consists in a pigmentation restricted to groups of cells, and is manifested (with an incidence of about 40%) with a delay of 3-5 generations, whenever an appropriate second chromosome in double dose is introduced into the genotype. The mode of inheritance--although bound to a chromosome--thus behaves in an unprecedented way in Drosophila.

Di Pasquale, A., and S. Koref Santibañez. Elements of fitness in tumorous and nontumorous stocks of D. melanogaster and D. simulans. Nine wild tumorous stocks (from near Palermo, Sicily) and 2 laboratory stocks, one with and one without tumors, of D. simulans, and 7 wild tumorous stocks (also from near Palermo) and 2 laboratory stocks (one with and one without tumors) of D. melanogaster have been analyzed with respect to fecundity, fertility, sterility, and developmental rate. From 2 wild stocks of simulans and 1 of melanogaster, showing tumor frequencies of 60%-80%, tumorous and nontumorous pairs of flies were selected and the elements of fitness determined quantitatively. No significant difference was found, except in developmental rate, which is faster in the progeny of tumorous flies. A comparison was also made between the tumorous and nontumorous stocks. No difference was found, even with regard to developmental rate, which, then, differs only between tumorous and tumorless individuals of the same stock. The maintainance of tumorous individuals in stocks of moderate tumor manifestation and of tumorous stocks in competition with tumorless stocks seems not to be based on differences with respect to the elements of fitness here studied.

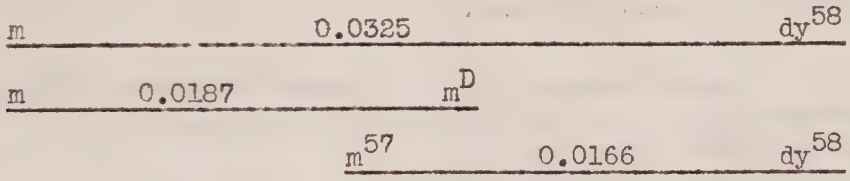
Divelbiss, James. Possible functional complexity at the bw locus in Drosophila. A spectrophotometric study of the red eye pigment was undertaken in all possible homo- and heterozygotes of bw^{59}, bw^{75}, bw^{81}, bw, and bw^{+}. Brown pigment was removed by including st in all genotypes. Measurements of AEA extracts were made with a Beckman DU quartz spectrophotometer over the region 220-560 mμ. Many of the heterozygotes showed gene interaction that is difficult to reconcile with a simple hypothesis of additive gene action. A wide range of E at 280/E at 430 ratios was obtained, indicating that the extracts contained at least two chemical entities, although it is not certain whether these entities were both actually components of the red pigment. One allele, bw^{59}, was unique in that AEA extraction did not remove all the pigment. The residual pigment was similar to the brown pigment left in the wild-type eye after AEA extraction. $Brown^{81}$ appears to produce a modified

red pigment. Studies of the possible structural, as well as functional, complexity of the bw locus are now being undertaken.

Dorn, G. L., and A. B. Burdick. Complementation and recombination at the miniature-dusky locus in D. melanogaster. We have constructed a map of the miniature-dusky region based upon suspected complementation. We assume that the wing length of a two-allele transheterozygote is determined by (1) the homozygous effect of the alleles themselves and (2) the degree of complementation between the alleles in heterozygous condition. The degree of complementation is assumed to be dependent upon the map distance of the alleles from each other. The effect of homozygous alleles was determined by measuring wing lengths. The expected wing-length value of any given heterozygote is the mid-parental value. This value is assumed to be equivalent to the effect of the genes themselves, assuming them allelic. We compared the observed values of the various heterozygotes with their expected values and used the difference as a measure of the degree of complementation. The diagram below shows a four-gene complementation map of this region, the numbers representing relative complementation values:



Recombination data collected during the past six months yields the following map for the region:



Ehrlich, Elizabeth. The pattern of sperm utilization by multiply inseminated females. Since it did not appear to be known with any certainty whether multiply inseminated females exhibit a nonrandom pattern of sperm utilization, and if so, what factors may influence it, experiments were undertaken to obtain further information. Utilizing stocks suggested by Dr. I. I. Oster, which permit one to distinguish the offspring arising from separate inseminations, females of various strains were mated to one type of male on one day, to another kind on the next day, and then allowed to oviposit until they had used up practically all the spermatozoa. Eggs deposited on each of the days following the second mating were kept separate, in order to determine the type(s) of sperms utilized by the inseminated females. Only F_1 females were included in the analysis of the data, since the presence of pre-existing lethals in the parent females might otherwise have led to erroneous conclusions.

The results, based on counts of more than a thousand offspring of

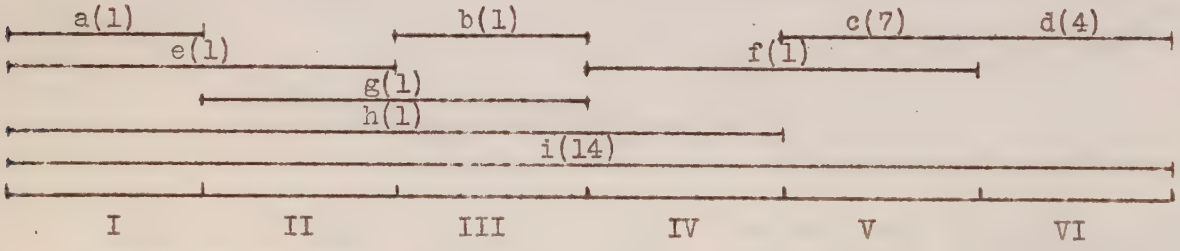
several females, indicated that the number of sperms used by any one female after mating varies, and does not seem to depend solely on the strain of the male (although strain differences no doubt exist) nor on whether the mating is of a virgin or of a nonvirgin mated on the previous day. The pattern which emerged is that females tend to utilize more sperm of the type which they have received (or retained) in larger quantity, as one would expect, but that it is utilized in a completely random manner.

It was also noticed during the course of these experiments that different strains of females vary considerably in the ease with which they will participate in mating on 2 successive days, and that of the 4 different strains used not one would mate on three successive days. That this may be related to the availability of space for sperm storage was suggested by the finding that lozenge females, which lack one of the 2 storage areas, the pair of spermathecae, would not mate on two successive days.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the U. S. Public Health Service, Contract RG-5286(2).)

Fahmy, O. G., and Myrtle J. Fahmy. Interallelic complementation in the r locus of D. melanogaster.

An analysis was undertaken of the phenotypic expression of crosses between 31 rudimentary (r) alleles. It soon became apparent that the mutants investigated constitute a pseudoallelic series involving several functional units (cistrons). Crosses between some of the r mutants produced an interaction F_1 heterozygote, expressing the rudimentary phenotype, whereas other crosses resulted in complementation in the F_1, producing the wild-type expression. It was possible to construct a complementation map (illustrated below) for the r locus, whereby the different mutants were arranged in a unique linear sequence involving different or adjacent cistrons.



C I S T R O N S

In the construction of this map, mutants which complemented were assigned to different functional units (cistrons). Thus mutants a, b, c, and d complement in pairs and therefore belong to different cistrons. This fact by itself, however, does not permit any determination of the sequence of the affected subgenes. The detection of the intralocus linear organization is made possible through the presence of mutants resulting from multiple "inactivations" (or damage), extending over more than one cistron. For example, mutant e interacts with a, but complements b; whereas h interacts with a and b but complements c. This fixes the position of b between a and c.

The whole of the complementation map suggests the presence of at least 6 cistrons in the r locus. The majority of the mutants seem to involve more than one functional unit. Nearly half (14 out of the tested 31) are of the

i type, involving all the 6 cistrons and giving noncomplementation to all other mutational types. These were at first thought to be small deficiencies at the r locus. Careful cytological analysis of a noncomplementing mutant (of the i type), however, failed to reveal even a single-band deficiency in the salivary X chromosome. According to the accepted criteria in Drosophila cytogenetics, therefore, even the multiple-cistron r mutants must be looked upon as intragenic point mutations.

A phenotypic manifestation that has some bearing on the complementation map is the appearance of the costal wing setae, or marginal bristles. In some intermutant heterozygotes the phenotype is wild type, except for the derangement and sparsity of the marginal wing bristles. This suggested a situation of "partial" complementation, which was designated (r+b) indicating an intermediate state between interaction giving the r phenotype and full complementation giving the r^+, or wild-type, expression. The analysis of the F_1 phenotypic expression in 92 interallelic crosses between complementing r mutants clearly showed that the "bristle effect" is an indicator of proximity. The F_1 heterozygotes of mutants damaged in juxtaposed cistrons gave the (r+b) effect in 45% of the crosses. This proportion showed a gradual decrease with the increase in the intermutant distance in cistrons. The (r+b) effect completely disappeared in crosses between mutants more than 3 cistrons apart.

The complementation map of the r locus in Drosophila is the most complete example so far ascertained in higher organisms. Evidence of intralocus complementation, however, has already been detected in our laboratory at 2 other X-chromosome loci, tan (t) and minute-chaete (mch), and doubtless it must occur in many others. Crossover experiments are now in progress to establish the spatial order of the r alleles. It would be interesting to elucidate the correlation between the functional complementation map and the linkage relationship among the subgenic alleles.

Falk, R. High spontaneous detachment of a y f:= ("double-X") chromosome.

Instability of the y f:= chromosome due to crossing over with another X chromosome in occasional triploids was reported a few years ago (Falk, DIS-28: 117, 1954). In an experiment with a y f:= stock, y^+ f^+ females were found with a frequency of 1 in 500-700. Of four such females analyzed, two proved to be triploids, one of which gave detachments, whereas the other two proved to be detachments due to crossing over with a sc^8 $Y^S.Y^L$ B^S chromosome that had been obtained from Lindsley. The detached chromosomes carried a recessive lethal; they were apparently composed of the proximal (inverted) X and a part of the $sc^8.Y^S$.

(This work was supported by a grant to H. J. Muller and associates from the U. S. Atomic Energy Commission, Contract AT(11-1)-195, and carried out while on a Public Health Service Postdoctoral Fellowship administered at Indiana University.)

Falk, R. Modification of dumpy by Moiré.

It has long been known (see Bridges and Brehme, 1940) that in the presence of Moiré heterozygotes for dumpy tend to show the oblique effect, and Carlson (Genetics 44: 347, 1959) found that this is true even of some alleles that ordinarily give only the lethal ('1)

or the vortex and lethal ('lv) effect in compounds. While working on one such mutant, thoraxate of Ives, dp^{txI}, an 'lv type according to Carlson's nomenclature, it was found that although no oblique effect appeared in the presence of the Mé InL InC e l_3e chromosome, it appeared with a high penetrance in the presence of the Mé Ins ri Sb^1 chromosome. To test this point further the Truncate allele, dp^T ('olv type) was crossed to the two Mé chromosomes, since this allele is known to give the strongest oblique effect in the heterozygote with Moiré. While the oblique effect showed with high penetrance and expressivity in the presence of the Mé Ins ri Sb^1 chromosome, there was low penetrance and very slight expressivity of the oblique effect in the presence of the Mé InL InC e l_3e chromosome. It has not been determined whether the increased penetrance and expressivity of oblique in the presence of the Mé Ins ri Sb^1 chromosome is attributable to the Mé mutant itself, in which case the Mé InL InC e l_3 e chromosome would contain suppressors, or whether the intensifying effect of the former chromosome is itself caused by genes other than Moiré.

(This work was supported by U. S. Public Health Service Grant RG-5286(2) to H. J. Muller and associates, and carried out while on a Public Health Service Fellowship administered at Indiana University.)

Falk, R., and Carol Bart. Exchange between an attached-X.Y chromosome and free Y chromosomes.

In an experiment to obtain an X.Y chromosome with visible markers on both ends, exchange in the male between Y^L of the Y^S.X InEN $y.y^L$ sc^8 y^+ chromosome and the corresponding arm of y^+ sc^8 $Y^S.Y^L$ B^S gave 4 y B^S progeny among 5987 males, whereas exchange in the male between Y^L of the resulting Y^S.X InEN $y.Y^L$ B^S chromosome and the corresponding arm of y^+ sc^8 $Y^S.Y^L$:bw^+ ("Cooper's Y") gave, most probably, 6 y males out of 8918 progeny (some small counts of different origin were excluded here; if they are included the exchange frequency is about 1/1000). Thus the over-all exchange frequency between the Y^L arms of these chromosomes is about 1/1500. No case of exchange between the Y^S of Y^S.X InEN $y.Y^L$ B^S and the corresponding arm of the y^+ sc^8 $Y^S.Y^L$:bw^+ chromosome was observed in 10,246 progeny (or, if the experiments of different origin are excluded, in 8918 progeny), as shown by the absence of the y^+ B^S progeny. This cannot be due to the previous loss of Y^S from the attached X.Y chromosome, since tests showed that it was present in at least most flies. Nor were any cases observed of exchange between the Y^S of y^+ sc^8 $Y^S.Y^L$:bw^+ and the Y^L of Y^S.X InEN $y.Y^L$ B^S, since no y^+ B^+ males were recovered. Another type of exchange, occurring between Y^L of y^+ sc^8 $Y^S.Y^L$:bw^+ and Y^S of Y^S.X InEN $y.Y^L$ B^S, could not be distinguished from the nonexchange types. These results are in apparent contradiction to earlier observations by a number of investigators that in cases of exchange between sc^8 X chromosome and a Y chromosome it is consistently the Y^S which participates in the exchange.

(This work was supported by a grant to H. J. Muller and associates from the Atomic Energy Commission, Contract AT(11-1)-195.)

Fujio, Y. Effects of some facet-forming substances on the eye discs of several B strains of D. melanogaster in tissue culture.

Recently it was found by several workers that various chemicals such as magnesium acetate, ammonium lactate, urea, and acetamide had some effect of increasing facet number in B flies of D. melanogaster when larvae were

raised on media containing them. It was difficult, however, to ascertain by the feeding method how these chemicals act on the eye. To settle the problem, eye-antennal discs and cephalic complexes taken from the mature third-instar larvae (95 hours after hatching at 25° C) of several B strains of D. melanogaster were cultured in vitro in synthetic media containing the chemicals in concentrations of 1 mM, 10 mM, and 100 mM respectively. The strains used were B (bb)-1, B (coiso), B; e^{11} (coiso), and bar-3 (coiso). Some chemicals, such as magnesium sulfate, ammonium lactate, urea, and acetamide, in concentrations of 1 mM and 10 mM, seemed to have a slight effect on differentiation of the ommatidia in eye-antennal discs cultured alone. By comparison with eye-antennal discs alone, eye-antennal discs cultured together with the cephalic complexes showed more complete differentiation of ommatidia.

Gahne, Birgitta. Drosophila species found in Uppland, Sweden.

The survey of Drosophila species at Uppsala, Sweden (M. Rasmuson, DIS-29, 1955) was continued during the summer of 1959. Flies were collected from the garden of the Genetical Institute near Uppsala, and also from different biotopes in the vicinity, such as pastures, forests, edges of forests, and shores of river and lake. The collections were started at the end of April and continued until the end of October. The first fly was caught May 15 (D. obscura ♂) and the last October 23 (D. obscura ♂). No collecting was done during July. Until the middle of August, paper cups hanging from trees with fermenting bananas as bait (after Spencer) were used as traps. During the rest of the collection period, modified Patterson cans (plastic vials covered by metal net) were placed on the ground, with fermenting bananas or pulp of raw apples as bait.

Fifteen different species were identified (see table). As the weather was unusually hot and dry, with only 104 mm of rainfall during the whole period as compared with the normal 300, the results may not be entirely representative of the normal populations. Especially the species that feed and breed on fungi--for example, D. transversa, D. phalerata, and D. testacea--must have suffered from the drought. The fact that D. phalerata was one of the most common species, and D. transversa very rare, indicates a difference in their breeding habits. D. transversa seems to be more dependent on fungi, and the same is probably true of D. testacea. D. alpina seems to require deep forests. D. silvestris was found mainly on the river shore but occurred also in the garden and forests. D. subobscura was the most common species in dry open pastures.

Seasonal fluctuation in contradiction to what was found in 1955 was revealed. Both D. subobscura and D. phalerata increased in relative numbers as the summer proceeded and reached a maximum at the beginning of September, at the same time that D. obscura reached a relative minimum. Some specimens belonging to other genera of the family Drosophilidae were found: Aulacogaster leucopeza (8), Amiota variegata (1), Amiota alboguttata (albilabris) (4), Parascaptomyza disticha (1), Scaptomyza gramineum (2), Chymomyza costata (11), Microperiscelis annulata (1).

(table on next page)

	Total	♀	♂	Total during each month: May	June	Aug.	Sept.	Oct.
D. nitens	13	1	12	2	4	5	2	
guyénoti	12	5	7			7	5	
busckii	8	3	5			3	2	3
melanogaster	610	292	318		8	193	373	36
subobscura	523	191	332	3	27	140	327	26
obscura	1087	530	557	52	120	374	457	84
silvestris	73	36	37	1	3	19	45	5
tristis	3	1	2			2	1	
alpina	29	22	7			3	26	
(obscura group, not det.)	40	21	19			11	24	5
transversa	18	8	10			11	7	
phalerata	332	141	191		6	79	243	4
(quinaria group, not det.)	8	5	3				7	1
littoralis	68	25	43		1	6	61	
testacea	8	4	4		1	2	5	
funebris	3	2	1				3	
hydei	1	1					1	
(not det.)	1	1				1		
Total	2837	1288	1549	58	170	856	1589	164

Glass, Bentley. The mutagenic effect of a 5 r dose of X-rays. An experiment is in progress to examine the mutagenic effect of a dose of X-rays at a level comparable to the 30-year background dose received by human beings. The use of dominant Minutes will eliminate the need for breeding tests of all F_1 individuals; and application of the X-ray dose to both male and female parents will mean that the effective dose measured in the F_1 mutation rate will be double the dose administered to any individual. From the published dosage curves for the induction of Minutes by X-rays in adult male and female D. melanogaster (Glass, 1955) it is estimated that the increase in frequency of Minutes for a dose of 10 r, assuming linearity with the dosage curve at 1000-2000 r, would be approximately 0.005%, in comparison with a spontaneous rate of 0.043%. Even to establish the significance of a difference at the .05 level of probability, and ignoring second order errors, it will probably be necessary to score at least 1 million flies in the treated series and 1 million in the controls; and even that number may prove to be inadequate. Results to date, however, are rather encouraging. Using coded methods so that the scorers do not know which series is the irradiated one in any replication, and controlling density of population and environmental variables so that variance within paired control and treated groups of the same replication is minimal and much less than variance between replications, we have scored a total of 11 replicate experiments, with the following results:

Minutes in unirradiated control series	99/230,251	0.043%
Minutes in irradiated series	114/229,500	0.050%

Almost exactly 50% of all Minutes obtained in both treated and control series either were completely sterile or were bilateral mosaics which failed

to yield Minute offspring. Seven Minutes in the control series and six in the treated series yielded sizable progeny without producing any Minute offspring. Thus 44% of the Minutes were genetically confirmed. By accepting these proportions of confirmed and unconfirmable Minutes as applicable to future series, we can speed up the scoring considerably in future months. It is of further interest that recessives, partial sex-linked dominants, and autosomal dominants confirmed by breeding have yielded the following data:

Visibles in control series	7/230,251	0.003%
Visibles in irradiated series	10/229,500	0.004%

Glassman, E. Allelism and complementation of bronzy (bz) and maroon-like (ma-l) eye-color mutants in D. melanogaster.

In DIS-32, Dr. M. J. Fahmy reported 5 alleles of a "new" eye-color locus called bronzy (bz) at 64.9 on the X chromosome of D. melanogaster. Dr. Fahmy kindly made available the allele induced by nitrogen mustard CB.3007.(the other alleles had been discarded); and biochemical and genetic tests have indicated that ma-l and bz are "allelic." No crossovers have been observed in approximately 5000 male progeny.

Biochemically, they appear to be superficially identical. Both have reddish-brown eyes, both are deficient in the enzyme xanthine dehydrogenase, and thus lack uric acid and isoxanthopterin, while hypoxanthine and 2-amino-4-hydroxypteridine accumulate. Transplantation experiments by Dr. H. Ursprung (see this issue DIS) show that bz, like ma-l, is nonautonomous. Finally, both are maternally affected if their female parent contains a wild-type allele (see Genetics 44: 547), and it is of interest that the progeny of a ma-l/bz female are not maternally affected (a functional test for allelism).

The eye-color of ma-l/bz is unexpectedly wild type, indicating that the deficiencies of ma-l are not exactly identical with those of bz, and one mutant can complement the functional losses of the other. This complementation is very slight, however. Although the eye color is wild type, the amounts of isoxanthopterin and uric acid are small by comparison with wild-type; and hypoxanthine, which is not easily detected in the wild type, is present in ma-l/bz flies. Furthermore, the enzyme xanthine dehydrogenase, which must be present in very low amounts, has thus far not been detected by an admittedly crude assay method. More sensitive methods are now being carried out. It is of interest that the 4 other chemically induced alleles of bz, found by Dr. Fahmy, do not complement.

Because of the allelism evidenced between ma-l and bz, we are renaming bronzy ma-l^{bz}.

The exact location of ma-l is still not determined. We were able to isolate f Bx^3 ma-l su-f flies from females which were f Bx^3 ma-l/su-f; this indicates that ma-l is to the left of su-f. However, su-f is reported at 64± (Bridges and Brehme) and Dr. Fahmy reported ma-l^{bz} at 64.9±2; thus additional data are necessary for the localization of these two genes, although the order seems to be Bx ma-l su-f.

composition of the medium, see the Technical Note by the author in this issue.

Movement of the Anlagen into their final position was observed. The formation of the chiasma externum is a result of the caudal-ventral movement of the medulla externa. Fixation of the border of the eye, formation of facets and ommatidia, their final differentiation, and the immigration of pigment all begin in the part of the Anlage next to the brain. Their determinations will be induced by the brain. The area of the ommatidia and the final size of the eye are initiated independently, the latter being determined by the genes for eye shape. B, ey, and L were studied.

The number of originally initiated facets is independent of the determination of the final number of ommatidia. Facets are always initiated over the whole ommatidia area, which is determined by the size of the head Anlage. Differentiation of the facets was observed. In the eye-shape mutants, retrogression of the facets between the bristle ring and final eye occurs simultaneously with the appearance of contact between the dioptic apparatus and the faceted covering membrane. The "unused" part of the faceted cornea becomes a covering epithelium for the original ommatidia area, similar to the rest of the head epithelium. The reaction capacity of the cells of the middle and outer layer of the imaginal disc decreases from caudal-dorsal to rostral-ventral.

The facet mutants gl, lz, and ro were studied. In gl the structural change can be recognized by the end of the second day of explantation, at the time of facet formation in the cornea, whereas in lz and ro the structural change does not take place until about the end of the third day of explantation. In gl and lz a smaller eye is formed. In these cases, too, the determination of the presumptive ommatidia area is independent of the final size of the eye.

The covering membrane is formed from the border cells of the Anlage at the same time as the rest of the head cuticula. In vitro, not later than 40-48 hours after explantation from the late third instar, within 12 hours a faceted membrane is formed from a structureless membrane, covering the entire presumptive ommatidia area. The opinion that corneagen cells (Hauptpigmentzellen) produce the cornea by secretion is not in agreement with our in vitro observations.

Grell, Rhoda F., and E. H. Grell. Correlation of primary nondisjunction of X chromosomes with the segregation of certain autosomes in oöcytes of D. melanogaster.

The discovery of Sturtevant (1944) that heterozygous autosomal inversions greatly increase the rate of nondisjunction of X chromosomes if they are also heterozygous for an inversion was further analyzed by Cooper, Zimmering, and Krivshenko (1955). They presented the hypothesis that when two different pairs of chromosomes are structurally heterozygous they may associate nonhomologously, and homologues may not always be directed to opposite poles of the first meiotic division spindle. Their data on the exceptional progeny and dominant lethals produced from oöcytes containing various inversion combinations are consistent with their hypothesis.

A desirable extension of their experiments is to demonstrate that when the X chromosomes undergo nondisjunction the autosomal chromosome involved in the nonhomologous association goes to the opposite pole from the two X

chromosomes. For this purpose, females of the genotype In(1)dl-49, y fa^n/y^2 v; T(3;4)86D/In(3R)Vno; + were produced and crossed to wild-type males. The following progeny were obtained:

	Regular	Exceptional		
		T(3;4)86D	Vno; +	Vno; 0 (haplo-4)
Females	1365	1	20	48
Males	1316	159	3	0

The translocation and the In(3R)Vno are obviously not recovered at random when there is X-chromosome nondisjunction. Nullo-X gametes tend to receive the translocation, and diplo-X gametes are usually recovered with the inversion. A sizable portion of the female exceptions are haplo-4. Generally we find that haplo-4 flies have a viability of about 1/3 that of wild type; therefore most of the female exceptional zygotes are actually haplo-4. Unfortunately the free fourth chromosome was not marked with a dominant mutant. If it had been, the majority of the exceptional males undoubtedly would have carried it and been recognized as triplo-4. Basing the calculation on males to avoid haplo-4 flies, primary nondisjunction occurred with a frequency of 19.8%.

Our interpretation of the data is that the inversion in 3R frees the part of the translocation that carries the distal part of 3R (from the break point at 86D) so that it may become involved with the X chromosomes during meiosis. (In(1)dl-49, y^2 v/y^2 v; T(3;4)86D/+; ci^D gives only 2.9% nondisjunction.) When the X chromosomes undergo nondisjunction it is because the translocation fragment has associated with one or both of the X chromosomes. The translocation fragment is directed to one pole of the spindle and the X chromosome with which it associated passes to the opposite pole. The free fourth chromosome is also often involved in the nondisjunctional process but not to the extent of the translocation fragment. We believe In(3R)Vno is pairing with the part of the translocation comprising 3L and 3R up to the break point at 86D, and is not actually involved in the nondisjunctional process. Zygotes that receive the inversion chromosome and any part of the translocation are lethal, and those that receive neither part of the translocation are lethal unless they receive the inversion chromosome; thus the nonrandom recovery of In(3R)Vno.

A similar type of experiment was one in which y^2 v/In(1)dl-49, y^2 v; T(3;4)86D/T(3;4)86D/ci^D females were mated to wild-type males. The following progeny were obtained:

Regular	Exceptional			
	Females		Males	
	ci^D	+	ci^D	+
3898	4	118	89	3

The frequency of primary nondisjunction of the X chromosomes is 9.9%. (From y^2 v/In(1)dl-49, y^2 v; T(3;4)86D/T(3;4)86D females without a free fourth chromosome 2.9% nondisjunction was calculated.) The free fourth

chromosome appears to interfere with the X chromosomes (probably only one X), and when the X chromosomes undergo nondisjunction the free fourth chromosome can be observed to go to the opposite pole.

Hertweck, H. Experimentally induced formation of a red pigment in the fat bodies and the Malpighian tubes.

Injection of distilled water and other hypotonic solutions into larvae of Berlin wild and some mutant stocks of D. melanogaster induces a red pigment, probably an ommochrome. The red pigment appears after metamorphosis in the cells of the fat bodies and Malpighian tubes. In Berlin wild and se, 40%, and in e^{ll} and w^{co} nearly 5%, of treated individuals produced such pigment. The mutant stocks w, w^{ch}, w^{a}, cn, and v were not able to form the pigment. Therefore this ability is concluded to be controlled by genetic factors. These data agree with the finding of Ursprung and his co-workers (1958), who showed an analogous effect induced by ultraviolet light in vitro and by transplantation.

Hoenigsberg, H. F., E. Gallucci, S. Giavelli, and G. P. Sironi. Sensitivity of pupal gametogenesis to X-rays.

Irradiation of 24-, 48-, and 72-hour old pupae of a Muller-5 stock was done with an X-ray machine delivering 220 kv at 12 Ma and with a 4-mm Al filter. The dose for 6 minutes amounted to 1360 r. The pupae were then allowed to develop; the males were recovered and crossed to 3-day-old Muller-5 females. Remating of each male to a fresh 3-day-old virgin female every 24 hours for 10 successive days provided the sperm batches needed to study the sensitivity of the pupae to X-rays. Dominant lethals were counted as the difference between the number of eggs hatched and the number of eggs laid by every female.

The results were used to test a hypothesis of work now in progress. Each cross was made 10 times, and counts of eggs laid and eggs hatched were compared. The same numbers of control crosses gave us the normal egg hatch. The results clearly demonstrated that 2-day-old pupae are the most sensitive. Whereas in the 24- and 72-hour-old pupae one batch was the most sensitive, the 48-hour pupae demonstrated sensitivity scattered over five consecutive sperm batches. Like the other pupal periods, however, they presented a peak of dominant lethals in the 5-6-day sperm batch.

It is of interest to note that many investigators agree that CO inhibits the cytochrome system. Inhibition, or simply low cytochrome oxidase level, is indicated by Bodenstein and Sacktor in second-day pupae of D. virilis. Other workers claim that CO may enhance the induction of mutations in an adenineless colonial strain of Neurospora crassa and attribute its effect to the reduction of molecular oxygen by the flavoproteins. Our results are very much in line with those of other workers, and may be explained tentatively on the basis of a reduction of cytochrome oxidase in the 48-hour irradiated pupae.

Horikawa, M., and Y. Kuroda. The in vitro cultivation of blood cells of D. melanogaster in a synthetic medium.

A new culture medium was devised for culturing blood cells of larvae of D. melanogaster in order to investigate the genetic and biochemical relations among single cells of various strains.

Third-instar larvae (about 80 hours after hatching at 25° C) of various strains grown in sterile conditions were transferred to the sterile synthetic medium on a depression slide. Blood cells were obtained by stabbing the larval bodies with a needle under a binocular microscope in a glass sterilizing chamber. When the blood cells obtained from ten larvae were dissolved in 1 ml of the synthetic medium, they showed an optimal concentration (about 500-1000 cells per mm^3). The suspension of blood cells was removed from the depression slide and transferred to roller tubes. The volume of medium in each tube was brought up to 1.0 ml, and the tubes were rotated at 1 revolution per 5 minutes at 25° C. One drop of cell suspension was pipetted from the culture tube every other day and treated with 0.2% Difco trypsin for hemocytometer count.

In the roller tubes the blood cells showed normal movement and mitosis for at least 2 weeks without a change of medium. The cells degenerated less rapidly in this improved medium than in the (K-6) medium previously used.

Recently, it was found that blood cells of third-instar larvae of Oregon-R increased 7.5-8 times in number in 8 days whereas cells of some tumorous strains increased only 3 times during the same period. This fact indicates that there may be differences among different strains of D. melanogaster in mitotic activity of the blood cells. The problem is being investigated.

Imaizumi, T. New lethal strains from the wild stock Miyazu of D. melanogaster. Four new lethal strains of very low hatchability were found in the wild Miyazu stock (collected in August 1958). The first was derived from outcrossing of Miyazu 1 ♀ x Oregon-RS 1 ♂ (1 among 3 pairs), the second from an X-rayed Miyazu male (3800 r), the third spontaneously from the first, and the fourth from inbreeding of Miyazu 1 ♀ x Miyazu 1 ♂ (1 among 8 pairs). The death rate in each lethal strain exceeded 50% of the eggs laid. Detailed studies of these strains are now in progress.

Jacobs, M. E. Relation of sex to dopa-oxidase activity in D. melanogaster. Colorimetric determinations of dopa-oxidase activity at 30° C in flies grown at 25° C disclosed that adult females showed very much more activity than adult males. Late-larval females (just before pupation) showed a higher rate than similar males, but the difference was less than that between male and female adults.

Kanehisa, T. Flavines, pteridines, and metal metabolisms related to the formation of melanotic tumors in Drosophila. Third-instar larvae of tu and v tu strains, raised on three synthetic media containing different quantities of riboflavine and xanthopterine, were analyzed qualitatively and quantitatively for flavines and pteridines by means of fluorospectrophotometry and spectrophotometry, and were also tested by paper chromatography and electrophoresis. Control samples underwent the same tests. The analyses clearly showed that flavin adenine dinucleotide (FAD) is intimately related to acceleration of tumor formation, showing an antagonistic relation to isoxanthopterine, which seems to have a positive effect on melanin formation. With reference to the previous data on metal analysis (Kanehisa,

1958), it is strongly suggested that FAD, together with molybdenum and probably rion, plays an important role in the mechanism of tumor formation.

Kato, M. Analyses of component fatty acids in D. melanogaster. The author reported in 1956 (DIS-30) the strain-specific pattern of fatty-acid content in D. melanogaster, and suggested that fatty acid plays some important role in metabolism and growth. Recently, the work was extended to separate analyses of the saturated and unsaturated fatty acids of pupal lipids. The lipids were mercurated and then the unsaturated fatty acids were separated by means of reverse-phase paper chromatography. Comparative quantitative analyses were carried out with the aid of the densitometer and by comparing extinctions of absorption spectra. In all the strains tested the following acids were commonly found: oleic (18 carbon atoms), palmitoleic (16), myristoleic (14), lauroleic (12), linolenic (18), linoleic (18), and arachidonic (20). According to the strain, the content of oleic and of palmitic acid varied with that of linoleic and of linolenic acid, respectively. On the basis of patterns of content of these unsaturated fatty acids, the strains were sorted into three groups: group A consisted of Tokyo, Oregon, Oregon-RS, Saikyo, and Canton-S; group B of v, cn, se, st, cl, and bw; group C of w, v bw, cn bw, and st bw.

By application of the bromazon reaction method, saturated fatty acids were distinguished from unsaturated ones. It was found that stearic acid (18 carbon atoms), palmitic (16), myristic (14), lauric (12), capric (10), caprylic (8), and butyric (4) were major constituents. These were commonly found in each strain, but there were group specificities as to quantities of the saturated fatty acids. Group A contained both stearic acid and palmitic acid in large quantities. Group B was conspicuous in having a large amount of palmitic acid and equal amounts of stearic and myristic acids. White-eyed strain of group C was easily distinguished by the presence of large quantity of these fatty acids in bulk. Data obtained from gas-liquid chromatographic analysis corroborated the results described above.

The differences in patterns of saturated and unsaturated fatty acid content of pupal lipids are very suggestive in connection with the metabolic pathways of lipids and the relation between genetic constitution of strains and pattern of lipid metabolism in Drosophila.

Khishin, Aziz F. Formaldehyde solutions applied by submersion. Chemical mutagens are usually mixed with the food of Drosophila larvae or adults, injected into their body cavities, or administered in the form of vapors. They can also be applied as solutions in which the various developmental stages of Drosophila, with the exception of the adults, are immersed (Khishin, 1956). It is suggested that this last method be called the "submersion method."

Tested for mutagenicity, aqueous solutions of formaldehyde applied by submersion proved to be successful in inducing recessive sex-linked lethal mutations in at least some stages of the male germ cells (Khishin, 1956 and unpublished). In addition to inducing mutations, the treatment affects the percentages of emergence and also the fertility of the emerging males (females not tested). Emergence is usually lower than that of controls, and there is a degree of sterility in the treated males, varying from a

slight lowering of fertility to complete sterility. The severity of these effects depends on the concentration of formaldehyde in the solution, on the duration of treatment, on the developmental stage treated, and, to a great extent, on the handling of the material after submersion. The purpose of this communication is to report the range of concentrations, and the periods of submersion, that can be used successfully for treating larvae and pupae of D. melanogaster with aqueous solutions of formaldehyde.

The three larval instars, prepupal, and three pupal ages were subjected to treatments. The ages were chosen to coincide with the appearance of the different stages of the male germ cells, or with different ages of the same germ-cell stage. Thus, calculated from the time of egg laying, the following were the ages treated: larvae, 40, 70, and 104 hours; prepupae, white stage, about 115-120 hours; pupae, 136, 160, and 184 hours.

The concentrations of formaldehyde solutions tested were 5, 10, 15, and 20 per cent. The duration of submersion varied from 15 to 60 minutes for larvae and prepupae, and from 1 to 4 hours for the pupal ages. The following concentrations and periods were found to be most suitable (i.e., gave reasonable emergence and fertility).

Larvae: 1st instar, 10% formaldehyde for 15 minutes. If necessary, 30 minutes may be used, but emergence will be as low as 15-20%. For the rest of the larval period, 10% up to 30 minutes, and up to 15% for 15 minutes.
Prepupae: 10% for 15-30 minutes.
Pupae: up to 15% formaldehyde for 3-4 hours.

With these concentrations of formaldehyde and periods of submersion, emergence is usually from 50% to 70% and sterility among the males is less than 20%.

Kikkawa, H. Genetical analyses of resistance to parathion in D. melanogaster.

From among many parathion-resistant strains of D. melanogaster, the following six, obtained from various localities, were analyzed genetically: Hikone (Japan), WMB (Japan), KSL (Sweden), SYSM-1 (U.S.A.), HLQ-2 (U.S.A.), and TG-57j (Korea). The experimental results showed that resistance to parathion in each strain was controlled mainly by a single dominant gene located at 64.5 on the second chromosome. In other words, the mechanism of resistance to parathion seems to be identical in different strains of D. melanogaster from different parts of the world.

King, R. C. Oögenesis in fu/fu- females.

It has been shown (Growth 21: 239) that flies homozygous for the gene fused are semisterile because of ovarian tumors, which increase in frequency as the flies age. About 50% of the oöcytes of 8-day-old fu females are tumorous. E. Glassman pointed out to me that chromosomes deficient for fused are produced when crossing over occurs between the ClB and y^4 inversions. Accordingly, crosses were made between In y^4, y^4 males and fu/ClB females to produce double-inversion daughters. These were crossed to fu males, and in the F_2 generation 11 fu/fu- females were obtained which were phenotypically fused Bar. None of these females laid eggs. Two females, sacrificed when 2 days old, showed no ovarian tumors. The rest, sacrificed when 7-8 days old, contained ovaries that were completely tumorous

with the exception of a few stage-14 oöcytes. Thus flies hemizygous or homozygous for fused have ovarian tumors, and the tumor frequency increases with age. Hemizygous flies, however, show a more extreme ovarian abnormality and are completely sterile. It thus appears that the gene fused is hypomorphic rather than amorphic.

King, R. C. Oögenesis in mr^2. The morula^2 females used in this study arose in a stock of genotype mr^2/Bld, In(2R)Cy obtained from the California Institute of Technology. Homozygous females and heterozygous males were grown together on cornmeal-molasses-agar medium at 25° C, and the reproductive systems of 14 females (6 to 7 days old) were examined as Feulgen-stained whole mounts. Oögenesis appears to be normal through stage 4. The compound, nurse-cell chromosomes then fall apart into their components, and these chromosomes condense to metaphase dimensions and then degenerate. The oöcyte karyosome also disappears. The result is the production of chambers with a normal envelope of follicle cells surrounding a cyst of sixteen Feulgen-negative cells. The largest chambers correspond in size to normal stage-6 oöcytes. Oögenesis was more regular in 1 of the 14 females. Here the oöcytes in stages 1 through 7 appeared relatively normal. Often, however, one or two of the fifteen nurse-cell nuclei behaved in the abnormal fashion described above. Stage-8 nurse-cell nuclei broke down into a series of Feulgen-positive droplets, which were carried into the oöcyte. Oöcytes in stages 9 through 13 were not observed, but stage-14 oöcytes were noted which contained Feulgen-positive spheres in their oöplasm. Morula females occasionally lay a few translucent, fragile eggs with chorionic appendages truncated or absent. Such eggs never develop into larvae.

Kitagawa, O. The effects of X-ray irradiation on selection response. Selection was conducted for large and small numbers of chaetae on the 4th and the 5th abdominal plates in an isogenic strain of D. melanogaster originating from strain Ore-R and designated the P line, and also in a hybrid between two isogenic strains, namely Ore-R and Samarkand, which was designated the C line. Selected parents in each generation were treated with X-rays (1500 r) just before mating. The high and low lines were classified in four lots, according to whether the treatment included (1) both sexes, (2) only females, (3) only males, or (4) neither sex. The selection intensity was 20% (6 out of 30 in each sex), except that no selection could be found in some later generations because of high sterility due to irradiation. After the 20th generation, lots 2 and 4 of the P line showed little or no response, whereas some response (a few units in chaetae counts) was obtained in lots 1 and 3. In the C line, a response was observed in all lots, an especially large response being obtained in lot 1. Lot 2 showed a response nearly equal to that of lot 3. These results seem to indicate that the remarkable effects of X-rays in inducing new mutations in polygenic systems and in increasing recombination, especially in females, may release already-existing genes. The response in lot 2 of the C line was strengthened by both effects, whereas in lot 3 the response was induced mainly by the former. Furthermore, the mutation rate in the polygenic system investigated under the influence of X-rays was thought to be higher in males than in females.

Lederman-Klein, Ada. A method for testing the influence of used culture medium.

One of the striking features of the homeotic mutant eyeless-ophthalmoptera (see J. Hered. 49: 262-266) is its penetrance, which exceeds that of most other multifactorial homeotic mutants. The increased penetrance of the ophthalmoptera protrusions observed in old versus fresh cultures might be due to maternal age, to changes in the culture medium, or to both. The frequencies of affected flies among the progeny of females aged for 2, 5, and 9 days before oviposition were 64%, 77%, and 80%, respectively, but the difference between mothers aged for 5 and 9 days was not significant ($P \sim 0.2$). The effect of used culture medium was tested by rearing ey-oph flies in bottles used previously by other strains.

Seven pairs of e or of y w flies per bottle were allowed to oviposit for 5 days and were then replaced by ey-oph parents aged 24-48 hours. Controls of ey-oph on fresh medium were started at the same time. After another 5 days all ey-oph parents were discarded. The penetrance of the homeotic phenotype amounted to 92.9±0.5% (N = 2645) in bottles used by e flies and to 97.2±0.4% (N = 4372) in cultures inhabited by y w, as compared with 84.7±0.5% (N = 4372) in the controls. Penetrance is thus shown to be raised by the conditions prevailing in the old medium. This method is more satisfactory than scoring old bottles, because of the poor yield of "second generation" flies which, furthermore, may overlap with flies of the first generation.

Lewgoy, F., A. R. Cordeiro, C. V. Tondo, and H. Winge. Chromatographic study of homozygous and F_1 intercrosses in D. willistoni.

Homozygous strains obtained from natural-populations samples from the El Destino Hacienda, La Plata, Argentina (6 homozygous for chromosome 2 and 7 homozygous for chromosome 3) and from Eldorado, R.G.S., Brazil (11 homozygous for 2 and 16 homozygous for 3), as well as 19 F_1 intercrosses of second-chromosome-homozygous strains and 15 F_1 of third-chromosome-homozygous strains were studied by two-dimensional paper chromatography. The first solvent was the upper phase of n-butanol--acetic acid--water, and the second n-propanol--1% ammonia (4:1:5 and 2:1 respectively). The most interesting results of the study of these 74 strains can be summarized as follows.

Isoxanthopterin spots were present for all the strains, but varied much more among the homozygous than among the F_1 intercrosses, these having "strong" intensity of this material. In the Eldorado homozygous strains, 3 second-chromosome and 7 third-chromosome showed "weak" or "medium" concentration.

Sepiapterin increased in the intercrosses of El Destino. The homozygous strains from this locality showed smaller amounts of this substance than those from Eldorado. Some strains homozygous for El Destino second or third chromosome lacked sepiapterin or possessed it in amounts below the limits of detection. These strains "weak" or lacking in sepiapterin also lacked the 11m spot of willistoni; that is, the Fl 7 of melanogaster; Hadorn and Mitchell, PNAS 37: 650, 1951). According to Forrest, Hatfield, and Van Baalen (Nature 183: 1269, 1959), the Fl 7 is the deoxy derivative of sepiapterin. Homozygosis for the second chromosome appears to decrease sepiapterin and Fl 7 more than does homozygosis for the third chromosome.

Biopterin occurred in all the strains mentioned here, and also increased in intensity among the F_1 individuals as compared with the P_1 homozygous strains.

2 amino-4 hydroxy-6 carboxy-pteridine and spot 6 (probably flavine adenine dinucleotide) were present in all the strains, but were weak and medium, respectively, in the homozygous strains, rising to medium and strong, respectively, in the F_1.

Several other fluorescent spots, not yet identified, exhibited the same increase. Some, very weak or apparently absent in the homozygous strains increased to weak, medium, or strong among the F_1, suggesting that the "new" "Hybrid substance" may be a result of the general increase in pterin content.

Lüers, H. The mutagenicity of triethylene thiophosphoramide (Thio-TEPA).

In order to study the influence of number of ethyleneimino groups on the mutagenic activity of a compound, and to determine whether or not the basic compound has any bearing on this action, experiments are in progress with ethyleneimino compounds of different compositions. Below are the results obtained in the Muller-5 test after feeding a solution of Thio-TEPA for 3 days under the same conditions as in former experiments with TEM and other ethyleneimino compounds. The administered concentration of 0.00023% (0.7 mg/300 cc) is the highest dose that allows a good yield of progeny (as in TEM). The results show that the mutagenic activity of the compound is of the same level as that of TEM, but clearly higher than that of 2,5-bis-ethyleneimino quinone, which possesses two ethyleneimino groups only. It is conspicuous, on the other hand, that in the brood pattern (3-day broods) there is a sharp decrease of rate in the 3rd brood, which is not observed in the experiments with TEM and with the quinone compound.

Brood	Chromosomes tested	Lethals No.	Lethals %
I	1227	92	7.45
II	843	57	6.76
III	1105	33	2.99

Lund, D. E. Further observations on the incidence of CO_2 sensitivity in North American species of Drosophila.

Laboratory strains of several other American Drosophila species, in addition to those reported by D. L. Williamson in DIS-31, have recently been found to have some sensitivity to CO_2. They are: D. algonquin (Lincoln, Nebraska); D. azteca (Durango, Mexico); D. macrospina (Albuquerque, New Mexico; Cross Anchor, South Carolina; Durango, Mexico; Halsey, Nebraska; Miller, Georgia; Seward, Nebraska); D. macrospina limpiensis (Patagonia, Arizona); D. pallidipennis (Bucaramanga, Brazil); D. prosaltans (Belem, Brazil; Baquete, Panama; and San Isidero de General, Costa Rica). Except for the Nebraska strains, there were kindly furnished by Drs. Marshall Wheeler and L. H. Throckmorton of the University of Texas.

Sensitivity can be induced in flies resistant to CO_2 by injecting them with an extract prepared from sensitive flies. The author has successfully induced sensitivity in a laboratory strain of D. macrospina (Seward, Nebraska) by means of an extract prepared from a CO_2-sensitive strain of D. melanogaster (Oregon). D. azteca (Durango, Mexico) and D. pseudoobscura (Hidalgo, Mexico) have also been made sensitive by injection with an

extract prepared from a sensitive strain of D. affinis (Weeping Water, Nebraska). The sensitive strains from which the extracts were prepared were kindly furnished by Dr. D. L. Williamson. Only in D. pseudoobscura has the sensitivity induced by injection been transmitted to the offspring. A 100%-CO_2-sensitive strain of D. pseudoobscura has now been established from the F_2 generation of the injected flies. The inheritance of the sensitivity is being investigated. Although CO_2 sensitivity has been found in a few wild D. pseudoobscura collected at Niobrara, Nebraska, it has not so far been possible to establish a stabilized CO_2-sensitive strain of this species by selection.

Hakino, S., and H. Takada. Drosophilidae from the Hidaka Mountain Group in Hokkaido.

A survey of Drosophilidae was undertaken in the Hidaka Mountain Group, August 1-10, 1959, using traps and some nets. The major part of the collection was made on Mt. Toyoni (1105 m high). A total of 664 specimens was obtained, comprising the following species: Amiota variegata, Leucophenga quinquemaculipennis, Mycodrosophila shikokuana, Drosophila alboralis, D. auraria Type A, D. auraria Type C, D. bifasciata, D. coracina, D. ezoana, D. funebris, D. histrio, D. histrioides, D. immigrans, D. lacertosa, D. moriwakii, D. nigromaculata, D. sexvittata, D. suzukii and D. testacea. They included two rare species, D. auraria Type C and Mycodrosophila shikokuana.

Malogolowkin, C. Transfer of the SR factor of D. equinoxialis to other species.

It was reported by Malogolowkin and Poulson (Science, 1957) that the "sex-ratio" factor of D. willistoni could be transferred by injection of cöplasm of abnormal eggs from "sex-ratio" females into adult virgin females from normal-sex-ratio strains. The same procedure is now being followed to inject abnormal eggs from "sex-ratio" females of D. equinoxialis into the abdomens of adult virgin females from normal-sex-ratio strains of the same species and of D. willistoni and other closely related species. Although the data are still few, there is a strong indication that the "sex-ratio" factor of D. equinoxialis can be transferred to females from normal-sex-ratio strains. Further studies are now in progress.

(This work was supported by a research grant from the National Research Council of Brazil.)

Mather, Wharton B. Additions to the Drosophila fauna of Australia.

The following is the summary of a paper in manuscript which has been submitted for publication. Illustrated descriptions are given of two new species from Australia, D. fumida of the Pholadoris subgenus and D. rubida of the immigrans species group, together with distributional records of 14 other species. The phylogenetic positions of the new species, in the light of anatomy, and hybridization tests and the evolution of their larval brain chromosomes, are discussed.

Mather, Wharton B. Cytological evolution in the immigrans species group.

The cytological evolution of the Australasian representatives (D. immigrans, D. setifemur, D. rubida,

and D. sp. E) of the immigrans species group is being investigated. D. immigrans has a metaphase plate of 3 pairs of rods and a pair of V's, D. setifemur 2 pairs of rods, a pair of V's, and a pair of dots. D. rubida 2 pairs of V's, and 2 pairs of rods, and D. sp. E 2 pairs of V's, a pair of rods, and a pair of dots. All have a giant-chromosome configuration of 4 long arms and a dot. These species will not hybridize; but, by a comparison of banding patterns among the species, 11 paracentric inversions have been detected (4 in 1, 3 in 3, and 4 in 2L). In D. rubida a strain from Rabaul (New Britain) has been crossed with a strain from Cairns (Australia). Using the Cairns strain as a standard, the Rabaul strain shows one simple and one complex inversion in the third chromosome. The inversion polymorphism of the group is being further investigated.

Mather, Wharton B. Drosophila survey of the Territory of Papua and New Guinea.

A preliminary survey of the Drosophila fauna of New Guinea was made in May 1959, utilizing banana baits. The areas sampled and the flies taken are listed in the table. Owing to the difficulty of accurately separating the females of the melanogaster species group, they have been listed as a group.

	Port Moresby	Goroka	Kavieng	Rabaul	Lae
Pholadoris subgenus					
D. novopaca ♂					
♀	1				
D. sp. A. ♂	1		11	2	4
♀	12		48	7	9
D. bryani ♂	51	3	6	6	14
♀	47	2	13	1	2
melanogaster group					
D. serrata ♂	11)	8)	5)	2)	)
D. pseudotakahashii ♂	1)	8)	)	)	)
D. ananassae ♂	259)	17)	162)	163)	26)
D. sp. B ♂	) 473♀	5) 62♀	) 237♀	) 187♀	) 16♀
D. sp. C ♂	17)	1)	16)	1)	19)
D. sp. D ♂	2)	)	)	)	4)
immigrans group					
D. sp. E. ♂	17	1	13	28	51
♀	25		5	34	49
D. rubida ♂	13	2	5	61	17
♀	23	1	15	22	25
D. setifemur ♂	106		2	41	3
♀	146		1	25	

Miyoshi, Y. Effects of some alkali metal ions on the development of D. melanogaster.

An interesting case of antagonism in terms of developmental hindrance was found between Na-ion and K- or Ca-ion. The strain-specificity of susceptibility to NaCl has already been reported (DIS-31, -32). The toxic effect of the agent upon developing flies seems to depend on the action of the positive Na-ion, not the negative Cl-ion. Both K- and Ca-ions produce a similar effect.

The emergence rates of bw (Kyoto) and Ore-R-S, the latter strain susceptible to NaCl, were tested with a culture medium consisting of 2 g agar powder, 10 g sugar, 10 g dry yeast, 0.4 g tartaric acid, 100 cc de-ionized water, and various concentrations of NaCl, KCl, or $CaCl_2$. In the control, which was reared with culture medium to which salts were not added, the emergence rate of eggs was 68% in bw and 73.6% in Ore-R-S. With culture medium containing NaCl at a concentration of 1.0 M the rate was reduced to 50.2% in bw and 2% in Ore-R-S. When a very small amount of KCl was added at a concentration of 0.05 M to the medium containing 1.0 M NaCl, the rate increased to 89.8% in the former and 55.8% in the latter (1.8 and 28 times the initial rates, respectively). A similar increase in emergence rate was also obtained with $CaCl_2$ instead of KCl; the rate became 89.0% in bw and 50.5% in Ore-R-S.

From these results it is surmised that the toxic action of high concentration of NaCl is counteracted by the addition of a small amount of either K- or Ca-ion, even though these are also toxic to developing flies when added in high concentrations. The effect of the Na-ion seems to be far greater than the effect, if any, of the Cl-ion.

Morita, T., and T. Tokuyama. Pigment in white-locus alleles.

In the white locus of D. melanogaster 10 or more alleles are known. Analysis of the genic action of these alleles should be useful for investigating the function and fine structure of the locus. The amounts of red and brown eye pigments in female flies of strains w, w^h, w^{bf2}, w^e, w^a, w^{co}, w^{col}, w^{sat}, and w^+ (Ore-r), and of heterozygous hybrids, were determined by the "double extraction" method. Results of the analysis are shown in the table.

	Red Pigment						Brown Pigment					
	w	w^e	w^a	w^{co}	w^{sat}	w^+	w	w^e	w^a	w^{co}	w^{sat}	w^+
w	0.0						0.0					
w^e	0.2	0.7					2.7	5.8				
w^a	0.5	0.9	1.3				2.0	7.4	6.0			
w^{co}	1.2	2.1	2.2	4.1			13.3	16.9	20.4	25.0		
w^{sat}	1.9	2.3	2.4	4.2	4.1		82.9	81.0	90.6	78.7	99.9	
w^+	73.9	70.6	66.9	65.5	68.3	100.0	88.7	78.7	92.8	79.6	88.4	100.0

On the basis of the results, the alleles of the w locus can be classified in four groups. The gene in the different groups is assumed to be a dissimilar unit of function.

Muller, H. J. A simplified breeding system for detecting sex-linked lethals in successive generations.

In the system of cyclic breeding for lethal detection that we described previously (DIS-26: 113-114), two types of males are used in alternate generations, and their X chromosomes zigzag. These males must be added from separate supply stocks since the male offspring of the test crosses are sterile (so that the females are automatically virgin). These complications are avoided in the scheme currently used, which employs parents of the composition y w^{m4},In/y In49 v B.Y^L ♀ and y^+ sc^{V1}.Y^S/y In49 v B.Y^L ♂. In each generation females of the type shown above are bred individually (those homozygous for v and B being discarded) to their fertile brothers. These fertile brothers are genetically like their father, the brothers with y w^{m4} being sterile for lack of Y^L. In case such males are absent from a given culture (because of a lethal or a small number of offspring) they may be supplied from other cultures. Thus virgins are unnecessary here. Moreover, individuals produced by nondisjunction, being recognizable, can be avoided in breeding. And in the rare cases of breakdown of the given X-Y system the individual breeding scheme, by giving evidence of extensive secondary nondisjunction, allows a prompt discontinuance of the aberrant line. This scheme affords a test for lethals in both the maternally and paternally derived X chromosome of each female bred, and after the first generation allows exclusion of all pre-existing lethals of the maternal line. Of course other inversions and markers could be substituted in place of those noted, with the exception of yellow, which is essential unless the less easily recognizable mutant achaete be substituted for it.

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Muller, H. J. An attached-X chromosome set-up of exceptionally high stability.

In order to allow the continuance of free competition between diverse X chromosomes of males for an indefinite number of generations, with a minimal risk of crossing over between them, it was necessary to devise a set-up in which males of the given types were always in the presence of females having attached-X chromosomes that could not, in effect, become detached and that were very unlikely, even if triploidy occurred, to engage in effective exchange with the separate X derived from the father. For this purpose the males were all provided with both the female-sterility genes singed(5) and ocelliless whereas the attached X's were provided with singed(x2) in one arm and ocelliless in the other, together with inversions to prevent unwanted crossing over, and lethals (at least one in each arm) to kill males receiving a detached X. One arm of the attached-X's was given the composition y ct^n oc ptg car and a lethal, induced for the purpose by Carlson, lying in the neighborhood of car, and the other arm was provided with y In49 sn^{X2} ct^{1},Inct. The last block of symbols represents a lethal cut allele that was induced in connection with a large inversion that probably has one break close to cut and the other in the proximal heterochromatic region, thus overlapping In49 and effectively preventing crossing over between the arms, except for a small amount to the left of In49. The attached X's having this constitution are designated by the abbreviation "snocty" while the separate X's of the males are all termed "snoc," with additional symbols for any other genes that require specification (e.g., "y snoc").

It will be noted that, although any males with detached X's die, any females with them are sterilized by the genes of the sn or oc locus. As for triploid females, crossing over could occur in them between the paternal X and the non-inverted arm of the attached X's. Females with attached X's derived from such crossing over, if it was to the right of sn, would be sterilized by sn, whereas if it was to the left of ct they would in effect retain their original composition; only in the very rare case of crossing over between ct and sn would there be a significant change, involving the acquirement of ct^+. As for males derived from such crossing over; if it was to the right of sn, as would happen in some two-thirds of the cases, they would become sn^+ but acquire ct^n. However, the fact that such males have never been found in the extensive examinations made of these cultures shows that a change in composition of the males caused by crossing over between the detached and attached X's of triploids occurs to a negligible extent.

This scheme of breeding is being used on a large scale in the experiments by Helen U. Meyer and the writer which test for the existence of invisible mutations affecting fitness.

(This work was supported by a grant from the U. S. Public Health Service, Contract RG-5286(2).)

Muller, H. J. Antimorphic behavior of Cataract. — So few cases of antimorphically reacting mutants have been found that each new one deserves being reported and investigated. Cataract eye, Cat, of Belgovsky (sometimes symbolized spa^{Cat} because of its allelism to L. V. Morgan's sparkling, spa) falls in this class. Lethal when homozygous, it produces a visible ommatidial "roughening" in the posterior portion of the eye in the heterozygote with a normal melanogaster fourth chromosome, but no visible effect with a normal simulans fourth in an otherwise melanogaster genotype (Muller and Pontecorvo, 1941). In melanogaster triplo-fourth (otherwise diploid) genotypes it produces more extreme roughening when there are two Cat and one normal fourth chromosomes, but is not lethal; the eyes are normal, however, when there are one Cat and two normal fourth chromosomes. Thus the degree of the phenotypic abnormality with melanogaster fourth chromosomes follows the graded series +/0 = +/+ = +/+/+ = +/+/Cat < +/Cat < +/Cat/Cat < Cat/Cat. That Cat is not simply a neomorph is shown by the normalizing effect of +, at least on the lethality, when added to Cat/Cat. It is of course possible that the competitive action is exerted by a competition for substrate (as Stern found to be the case with some cubitus alleles) rather than by the induction of a reaction actually antagonistic to that induced by the normal allele. In this case the reaction induced by Cat could be at the same time a neomorphic one, since it is not visibly affected by changes in the number of the + alleles unless Cat itself is present. It may be that dominant cubitus, ci^D, falls in this category also. If so, however, it has a different level of penetrance, inasmuch as we find that, unlike Cat, it produces a markedly mutant phenotype (of cubitus expression) when in company with two non-cubitus fourth chromosomes in triplo-fourth diploid melanogaster. These differing properties make the mutants useful in reciprocal ways in tracking the segregation of fourth chromosomes.

(This work was supported by a grant from the U. S. Public Health Service, Contract RG-5286(2).)

Nolte, D. J. Retinular pigments.

Adults and pupae of D. melanogaster and D. pseudoobscura have been studied by means of the electron microscope to obtain a clearer picture of eye structure and pigment granule growth. The red pigment or drosopterin is found in the distal parts of the secondary pigment cells without any admixture of the brown pigment, but also in the basal parts of the secondary cells and in the postretinal region, where a mixture of pigments occurs. The brown pigment or ommochrome occurs as the only pigment in the primary cells, but is mixed with the red pigment in the secondary cells and in the postretinal region. The rhabdome consists of six normal retinulae whose bases, on the basement membrane, are dilated into bulbs blocking the lower end of the rhabdome, and a seventh or displaced retinula whose basal end has electron-dense cytoplasm and is in close connection with the nucleus of the eighth retinula, which is much reduced. In the distal tips of the seven retinulae small granules of the ommochrome cluster around the rhabdomeres. In the dilated bases of the six normal retinulae, granules of this pigment, twice the size of the former, occur in large numbers. In the base of the seventh retinula, in the electron-dense cytoplasm, a layer of granules of the same pigment occurs, the size being intermediate in comparison with the other two types of granules. About twenty eye-color mutants have been studied submicroscopically in D. melanogaster and four in D. pseudoobscura. These retinular granules may be absent in some mutants, but generally their sizes do not vary in correlation with different amounts of ommochrome.

Ogita, Z. An attempt to reduce and increase insecticide resistance in D. melanogaster by selection pressure.

Further experimental results with phenylthiourea and phenylurea selection pressure supported the hypothesis presented in previous papers (Botyu-Kagaku 23: 188, 1958; Nature 182: 1529, 1958), that phenylthiurea selection pressure can eliminate a dominant gene (2-65±) for resistance to DDT, BHC, and parathion, and restore these insecticide susceptibilities in surviving flies, whereas phenylurea pressure can restore these insecticide resistances in surviving flies, and that both pressures can produce nicotine sulfate-resistant flies. DDT susceptibility restored by one or several selections with 10 mM phenylthiourea was not so high as that of Canton-S (extraordinarily susceptible strain in D. melanogaster) but came close to it.

Ogita, Z. Toxicity of phenylthiourea and phenylurea derivatives to several D. melanogaster strains resistant or susceptible to insecticides.

In previous papers, it was reported that resistance to DDT, BHC, parathion, and phenylurea is negatively correlated with resistance to phenylthiourea. Some chemicals were tested to determine whether they have the same peculiar property as phenylthiourea. It was found that p-halogen substituents of phenylthiourea, such as p-chlorophenylthiourea and p-bromophenylthiourea, are negatively correlated with DDT, BHC, parathion, and phenylurea, and that p-halogen substituents of phenylurea are positively correlated with these insecticides.

Oshima, C. Genetic variability in resistance to DDT and to dieldrin in wild-type strains of D. melanogaster.

It is assumed that natural populations are heterogeneous for factors for resistance to DDT and to dieldrin and that the expression of resistance is normally distributed. Using 4% DDT test papers, the levels of resistance were measured for 27 strains collected from different localities in Japan, for four strains collected in foreign countries, and for 3 isogenic strains. Dosage-mortality regression lines for the different strains were estimated from cumulative mortality data after exposure of the flies to the test papers for 8 and 16 hours. Some of these regression lines were flat, indicating heterogeneity in the polygenic system; others were steep, suggesting greater homogeneity. Tests for resistance to dieldrin carried out on several strains with 0.4% test papers gave similar results. As another measure of genetic heterogeneity, counts were made of the numbers of bristles on the fourth and fifth abdominal sternites of 100 female flies from each of 23 wild strains--a character assumed also to be under polygenic control--and the coefficients of variation were computed. If two aberrant strains were excluded, for the remaining 23 strains a highly significant negative correlation was obtained between the slope of the dosage-mortality regression line and the coefficient of variation of the sternal bristle numbers.

Oshima, C., and O. Kitagawa. Effects of induced lethals on viability in heterozygous condition.

Male flies of an isogenic Samarkand strain of D. melanogaster were irradiated with 500 or 1000 r of X-rays, and twenty-seven second-chromosome lethals were found by testing with M-5; Cy/Pm; Ubx/Sb. Each lethal gene was maintained in balanced condition with the Cy inversion and the other two major chromosomes were replaced by nonirradiated ones from the original strain by appropriate matings. Thus the genetic backgrounds of these flies having different lethals were almost uniform. Before testing for viability of the lethal heterozygotes, localization tests were carried out. It was not definitely established that each lethal was due to a single point mutation, but twenty-six lethal genes were presumed to be at different loci. Two lethals, l_{13} and l_{20}, were identical. Samarkand male flies were mated to Cy female flies of these balanced lethal stocks, to test the viability of lethal heterozygotes, and the numbers of offspring (Cy/+ : l/+) from several replicated cultures were scored and compared. The percentage of lethal heterozygotes (l/+) was expected to be 50, but the over-all average percentage was estimated to be 46.3024. The percentages of two lethal heterozygotes were over 50, but not significantly, and the percentages of six lethal heterozygotes were significantly below 50. These percentages should be corrected by the ratio of Cy/+ and +/+ flies in the F_2 offspring. The results show that almost all the lethal heterozygotes had lower viability than the original isogenic flies. Experiments for determining the degree to which individual lethals from natural populations lower viability when they occur in heterozygous condition are being carried out by Pearl's method.

Paik, Y. K. Further studies of lethal concentration in a Korean population of D. melanogaster.

Some results of preliminary tests on this subject, made with 1957 collections, were summarized in DIS-32, pp. 145-147. Around the end of August 1958, another collection was made at one of the three localities, Najoo.

This population sample is designated NJ-58h in the following descriptions. From tests of a total of 139 second chromosomes, the following data were obtained:

Combined frequency (le. + semi-le.): 7.91±2.29%

Lethal allelism rate (%): 41.67±8.21

Comparison of the NJ-57i and NJ-58h data shows that the lethal frequency dropped approximately 5.5%, and the amount of allelism increased by about five times in 1958. The statistical differences are significant, not in frequency ($P = 0.131$) but in amount ($d/SE_d = 5.22$, $P < 0.0001$). The occurrence of such drastic annual change is interpreted by the author as being due to the effect of genetic drift, which can operate in a small population. The data strongly suggest that the Najoo population had not been breeding successively large enough through the years, but might have been reduced greatly in size, periodically and occasionally, during the period. This hypothesis may be further supported by the results of allelism tests between the 17 lethals from NJ-57i (one stock was lost) and the 9 lethals from NJ-58h, in which no alleles were detected.

These findings give added support to the conclusion that the breeding structure of *D. melanogaster* populations in Korea provides a rather interesting case in which the lethal mutation load is very low, perhaps the lowest, as compared with that reported so far for the second chromosome of this species or the equivalent chromosome of other species.

Paik, Y. K. Nonrandom distribution of lethal genes in the second chromosome of *D. melanogaster* in natural populations.

All the lethals (including identical ones) derived from the NJ-57i, TG-57j, and QI-57i collections (see DIS-32: 145-147)--43 in all--were tested for localization in the second chromosome. Preliminary localization of each of these lethals was accomplished by the use of a marker chromosome carrying the two dominant genes Bl (54.8) and L^2 (72.0). After this initial test, which showed the presence of the lethal gene in either the left or the right arm, the lethal chromosome was tested a second time, or a third when necessary, with either one or both of the following two marker chromosomes: Pfd (70.8)--Pin (107.3), Sp (22.0)--J (41.0).

Among the total of 28 different lethal chromosomes (excluding the identical alleles), 12 represent "multi-locus lethals"; 1 is a chromosomal lethal (deletion); and 15 are "single-locus lethals." The most remarkable fact noted is that all the single-locus lethals recovered are either in the right arm or near the centromere region, none in the left arm outside the centromere region. The distribution of single-locus lethals is apparently not a matter of chance alone in these populations. Even when the multi-locus lethals are taken into consideration, a theory of nonrandom distribution seems justified. This view is further strengthened by examination of the loci of the visibles recovered in the three populations. Except for four new mutants (unlocalized), only one is in the left arm, 3 are in the right arm, and the remaining 8 are near the centromere region.

Thus the present data show that the distribution of lethals in the second chromosome is not due solely to chance but is concentrated either in the right arm or near the centromere region. Details will be published elsewhere soon.

Pipkin, S. B. Time of development of sexual forms in D. melanogaster.

Dobzhansky's (1930) experiment determining the time of hatching of the various types of progeny of triploid females at 27° C was repeated at 22±0.5° C. Fertilized y^2; ru ca triploid females were allowed to lay eggs during a 2-hour period and then removed from the bottles. A count was made of flies hatching every 2 hours for 4 days; every 6 hours for 2 days, and once on the 8th and 10th days. Beginning with the appearance of the first imagines 244 hours later, the sexual forms emerged in the sequence indicated by the following mean hatching times, stated in number of hours after the end of the egg-laying period: diploid female, 287.17±0.63; triploid female, 295.33±0.35; diploid male, 303.53±0.64; intersex, 331.05±0.81; superfemale 365.89±5.50; supermale, 406.00±9.12. Mean developmental time was 87.93 hours longer for diploid females at 22° C than at 27° C (Dobzhansky's study), and correspondingly longer times were required for other sexual forms. Although the order of hatching of the various sexual forms at 22° C was similar to what Dobzhansky found at 27° C, one distinct difference in development at the two temperatures was observed. At 22° the earliest-hatching intersexes (2X3A) emerged only 24 hours after the first diploid females and 24 hours before the median hatch of diploid females. All sexual forms continued hatching to the 10th day after first emergence of imagines. At the higher temperature (27°) Dobzhansky found that practically all the individuals representing balanced chromosomal sexual forms (diploid females, 2X2A; triploid females, 3X3A; diploid males, 1X2A) hatched first. Then for a short time scarcely any flies emerged until the intersexes, followed by the supersexes, began hatching. Hence the lower temperature (22°) is a more favorable environment even for the unbalanced intersexes.

The percentages of the different sexual forms developing at 22° as progeny of triploid females are as follows: diploid females, 38.54; diploid males, 18.96; triploid females, 12.84; intersexes, 28.66; superfemales, 0.50; supermales, 0.42. These percentages agree rather closely with those found by Dobzhansky.

*Prevosti, A. Natural breeding site of D. ambigua Pom.

In February 1958, two pupae of D. ambigua were found in slime flux of Quercus ilex, in a wood of this typically Mediterranean tree, in Vallvidrera near Barcelona. In March 1959, another pupa and six advanced larvae, which developed into adults of D. ambigua, were found in slime flux of another specimen of Quercus ilex in the same wood. It is interesting to notice the ecological similarity of the breeding site of the European D. ambigua to that of D. pseudoobscura, since D. ambigua is the only European species of the obscura group that crosses with D. pseudoobscura.

Prout, Timothy. Correction for maternal influences in population sampling.

Maternal influences on quantitative characters constitute a disturbing factor in the study of genetic differences among sets of gene pools derived from natural populations. One solution is to make reciprocal crosses between the populations being studied, thereby obtaining a quantitative estimate of the maternal influences involved, which in turn permits correction.

For example, the table shows a set of wing-length determinations on the female progeny of D. pseudoobscura collected at Prescott, Arizona and Keen

Camp, California and on the reciprocal F_1's between them. The difference between the P_1 means is .8 mm x 100, which value does not differ significantly from zero (t = 1.15). However, the difference between the reciprocal F_1's is 3.9 mm x 100, which does differ from zero (t = 4.27, DF = 50), thus indicating maternal influences. Assuming the maternal influences are acting on the P_1's as well as the F_1's, and taking into account the direction of this action, in this case the "true" difference between the P_1's should be their observed difference plus the difference between the reciprocal F_1's or .80 + 3.90 = 4.70 mm x 100. Since this latter figure is the sum of two differences involving all four original means, its sampling variance is the sum of the sampling variances of the individual means (1.3190). A "t" value may then be derived, testing the hypothesis that the value 4.70 mm x 100 does not differ statistically from zero. The value so obtained is t = 4.25 (DF = 128), which is significant. Thus, if reciprocal crosses had not been made, the gene pools of Prescott and Keen Camp would have been judged identical, whereas the correction for maternal influence revealed them to be different. The writer is in possession of some data in which the maternal influences actually reverse the apparent relationships of the P_1's.

Female wing lengths in mm x 100 of populations of D. pseudoobscura and their crosses derived from Prescott, Arizona (P) and Keen Camp, California (K)

Female Parent			Male Parent	Mean	S^2	N	S^2/N
P_1	P	x	P	158.3	10.70	43	.2488
P_1	K	x	K	157.5	8.67	37	.2343
F_1	P	x	K	156.0	9.56	23	.4156
F_1	K	x	P	159.9	12.19	29	.4203
							1.3190

Ray-Chaudhuri, S. P. and A. S. Mukherjee. Mutagenic effect of quinhydrone on melanogaster and ananassae.

Experiments were designed to detect visible mutations in D. ananassae and sex-linked lethals in D. melanogaster after treatment with aqueous solutions of quinhydrone of different concentrations, namely, 10^{-3} M, 10^{-2} M, 10^{-1} M, 1.6×10^{-1} M, 2.0×10^{-1} M, and 2.5×10^{-1} M. The quinhydrone solutions were mixed with the medium in the proportion of 20 cc of quinhydrone to 200 cc of medium. Stocks of only twelve different visible mutations have been established from the mutations obtained. Others could not be used owing to low viability or fertility of the mutants. Four of them are sex-linked and the others autosomal. Some of these mutants were recurrent in the same and in different experiments, thus yielding a high frequency of visible mutations.

The total frequency of sex-linked lethals and semilethals in melanogaster, tested by the ClB technique, was approximately 4%-6%, and the frequency of visible mutations in D. ananassae ranged from 8% to 15%. The variation in the rate is in no way correlated with the doses of quinhydrone in the different experiments. The control series gave a frequency of 0.03% to 0.13% for sex-linked lethals in melanogaster and only 0.3% to 0.4% for visibles in ananassae (see the table). The mutations obtained by this treatment are being reported elsewhere.

Stocks Treated	Conc. Quinhydrone	No.males tested	No.chromosomes tested	Sex-linked lethals No.	Sex-linked lethals Freq.	Sex-linked semilethals No.	Sex-linked semilethals Freq.	Visibles No.	Visibles Freq.
Oregon-R	10^{-3} M to $2x10^{-1}$ M	100	2410	10	.4%	86	3.6%	31	1.3%
Oregon-R	Control	50	1231	1	.08%	0	0	0	0
Howrah +	10^{-3} M to $2x10^{-1}$ M	100	1291	12	.9%	0	0	144	11.1%
Howrah +	Control	50	831	0	0	0	0	3	.36%

Röhrborn, G. Mutation tests with melamine and trimethylolmelamine.

Triethylenemelamine (TEM) and trimethylolmelamine are both derivatives of melamine. For purpose of comparison, melamine and trimethylolmelamine were fed for 3 days to males by the same method used by Lüers (1953) for testing the mutagenic activity of TEM. Melamine applied in the form of a 0.0023% aqueous solution and as a 1% aqueous suspension did not show any effect in the Muller-5 test (8/2065 = 0.39%, and 8/5304 = 0.15%). Trimethylolmelamine gave corresponding results when administered as an aqueous suspension of 0.0023% (4/1131 = 0.35%). Feeding this substance in the form of a 1% suspension produced the following results:

Brood	Chromosomes tested	Lethals No.	Lethals %
I	378	20	5.29
II	439	16	3.64
III	260	10	3.85

The lower mutagenic activity of trimethylolmelamine compared with TEM may be explained by the fact that the former compound is less soluble, or that it possesses rectilinear oxymethylamino groups instead of cyclic ethyleneimino groups (TEM). The basal compound, melamine, seems to have no share in the genetic activity of its two derivatives.

Sang, J. H., and R. C. King. Nutritional requirements for normal oögenesis in D. melanogaster.

The nutritional requirements of axenically cultured D. melanogaster larvae have already been defined. It was anticipated that adult requirements which would permit full fertility of females would be similar; although it is well known that adult survival can be maintained on sugar-water alone.

Axenically reared flies were transferred under sterile conditions to 6-x-1-inch tubes containing the test diet as a fluid in a nonabsorbent cotton base. After 4 days of feeding, females were transferred to 3-x-1-inch vials stoppered with a cork having a disc of agar on which the eggs might be laid. Egg production was then assessed by counting the eggs laid

during a 24-hour period, and egg viability by counting the eggs that had failed to hatch after a further 24 hours of incubation at 25° C. The observations were repeated after the adults had fed for 8 days (after which time they were transferred to fresh food), and again after 16 days' feeding on the synthetic diet, whenever this was necessary. A standard larval food medium (Sang's 1955 medium C) was taken as prototype, and the qualitative requirements of the adults determined by omission of individual constituents. The standard complete food gave a productivity (number of viable eggs) similar to that found with live yeast, so that effects of omission can be expressed as a percentage of this standard.

The results may be summarized as follows. The figures in parentheses show productivity after 16 days' feeding.

Nutrient omitted	Productivity on 9th day	Nutrient omitted	Productivity on 9th day
1. arginine	4%	17. thiamine	100% (100%)
2. histidine	12%	18. pantothenic acid	100%
3. isoleucine	0	19. nicotinic acid	2%
4. leucine	0	20. folic acid	15%
5. lysine	0	21. riboflavine	5%
6. methionine	11%	22. pyridoxine	2%
7. phenylalanine	0	23. choline	100%
8. threonine	0	24. biotin	100%
9. tryptophan	0	25. cholesterol	100% (100%)
10. valine	0	26. fructose	100%
11. glycine	18%	27. RNA	100%
12. glutamic acid	100%	28. K	0
13. serine	100%	29. P	14%
14. proline	18%	30. Mg	0
15. cystine	7%	31. Ca	100%
16. alanine	6%		

The most surprising of these results are the failure to demonstrate an adult requirement for thiamine or for cholesterol, both of which are essential for larval development; the apparent lack of requirement for pantothenic acid; and the failure of omission of RNA to affect fecundity or fertility.

The ovaries of flies fed diets lacking isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, methionine, nicotinic acid, folic acid, pyridoxine, riboflavine, K, Mg, or P lack oöcytes in stages 8-11. Since these are the stages during which yolk synthesis occurs, it appears that these nutrients are essential for vitellogenesis. Pyridoxine-deficient ovaries show additional abnormalities which involve the oöcyte, nurse cells, and follicle cells. Oöcytes are often located in the center of an egg chamber, and whether this abnormal position is the result of active migration or passive displacement is not known. The growth of the various elements of a chamber may be retarded to different degrees. For example, a chamber of volume equivalent to stage 8 may contain a stage-7 oöcyte and nurse cells whose nuclear volumes correspond to stage 9. The follicular epithelium often contains pycnotic cells, although the nurse cells are morphologically normal. The epithelium may hypertrophy in a localized area, or clusters of follicle cells may enter the interior of the chamber. Nurse-cell nuclei are often deficient in Feulgen-positive material in a manner

reminiscent of sn^{36a} and fs 2.1 nurse nuclei. As was mentioned by us in DIS-32 (p. 132), Mg is required for yolk synthesis but not for chamber proliferation. The other 14 nutrients listed above are required for chamber proliferation and for maintenance of preyolk chambers and germaria. Prolonged omission of any of these factors from the diet leads to degeneration of the posterior oöcytes in each ovariole. Nurse cells degenerate before follicle cells. Eventually each ovariole may contain only 2 or 3 oöcytes. The germaria become spherical, contain no germarial cysts, and show no mitoses and little cellular detail.

(This study is part of a collaborative program carried out during the tenure of R. C. King of a National Science Foundation Postdoctoral Fellowship while on leave from Northwestern University.)

Sävhagen, Ruth. A new method of studying frequency of induced genetical damage and at the same time the treated cell stage.

In mutation work with X-rays and chemical mutagens, several attempts have been made by various indirect methods to correlate the sensitivity period with the treated cell stages. This is a more direct method to study frequency of induced genetical damage and at the same time the treated cell stages. The indication of genetical damage adopted for the experiments was occurrence of XO males, and the criterion of early meiosis (prior to anaphage I) was the induction of nondisjunction between X and Y in the males.

The females were taken from y w sn ♀♀ x y w sn; sc^{8Y} ♂♂, and the males from y^{16} ♀♀ x y^{16}; sc^{8Y} ♂♂. Normal offspring would give y females and w sn males. Individuals that had lost X or sc^{8} Y, and consequently were XO, would phenotypically be y w sn; and eggs fertilized by an X; sc^{8} Y-carrying sperm (nondisjunction) would give wild-type females (y w sn; y^{16}; sc^{8Y}). As such females may also arise through induced crossing over between the X and Y chromosomes, each wild-type female was tested individually. The majority were due to nondisjunction. The males were irradiated with 1100 r in air when 0-1 or 3-4 days old. Immediately after irradiation the males were transferred to a mating box. The first mating period lasted 4 hours, but from the 4th day onwards the mating periods were 24 hours long. The results are shown in the table.

There was a sharp increase in frequency of wild-type females on the 7th day after irradiation. The frequency reached a peak during the 8th day, after which it dropped. From this it may be concluded that the broods from the 7th to the 11th day after irradiation correspond to early meiosis (prior to anaphase I). From the table it is also evident that, regardless of the age of the male at the time of irradiation, the peak of nondisjunctional females will occur in the same brood. In both experiments the number of XO males rises steeply from the 4th brood, reaching a peak on the 8th day after treatment and then decreasing towards the 11th day. Thus there is agreement between the highest X-ray sensitivity and cells treated in early meiotic stages. But there are also points of disagreement, as a high frequency of XO males is obtained during the 4th to 6th days after irradiation, when the rate of nondisjunctional females is at the control level. Experiments on this problem are under way.

Mating period after irradiation	0-1-day-old males			3-4-day-old males		
	% XO ♂♂	% Non-disj. ♀♀	Total offspring	% XO ♂♂	% Non-disj. ♀♀	Total offspring
0-4 hours	0.46	0.01	20,538	0.32	0.03	44,635
4th day	0.33	0.03	62,692	0.20	0.04	43,016
5th day	0.75	0.03	50,552	0.43	0.02	38,766
6th day	1.33	0.01	24,735	0.68	0.03	17,818
7th day	2.21	0.22	18,100	1.24	0.12	14,155
8th day	3.47	0.51	13,350	1.55	0.21	11,706
9th day	2.39	0.19	7,772	0.96	0.13	8,724
10th day	0.54	0.18	27,946	0.49	0.14	8,312
11th day	0.46	0.14	30,044	0.19	0.05	7,807

Scharloo, W. Expression of ci^D at different temperatures. Uniform adaptive reactions by wild-type populations are thought to originate through natural selection which favors, within a population of individuals reacting in different ways, those that exhibit the most useful type of reaction. There is support for this hypothesis in the fact that wild populations do not react uniformly to abnormal environmental conditions. On the strength of this view one might expect that the same experimental change in environment would result in diverse reactions in different individuals or populations, as would also an abnormal genetic situation such as that produced by introducing a mutant into a wild genotype. An experiment was carried out by introducing the balanced fourth-chromosome mutants ci^D/spa^{Cat} into three wild stocks recently obtained. Flies were reared at 20° and 25° C, and the expression (E) of ci^D was measured as the percent ratio of length of fourth to third vein distal of the anterior crossvein. The results, shown below, were obtained by measuring the left wings of 40 females and 40 males in population 1 and 60 females and 60 males in populations 2 and 3.

	E in females				E in males			
	25°	20°	25°-20°	P	25°	20°	25°-20°	P
1. Steenbergen	52.3	56.6	-4.3	< 0.001	46.6	50.4	-4.2	< 0.001
2. Kolmar	49.1	48.0	-1.1	> 0.4	40.5	39.8	+0.7	> 0.14
3. Leiden	43.7	34.7	+9	< 0.001	32.2	29.3	+2.9	< 0.001

The presence and direction of the reaction of a measurable wing character dependent on ci^D is clearly a property of the developmental system influenced by ci^D rather than of the mutant gene itself. Thus the reaction is entirely dependent on the genetic background.

Seto, F. Respiration curves of some pupal lethals. Data of a preliminary nature have been obtained regarding oxygen consumption in some "pupal" lethals. A simple capillary respirometer was used to measure the oxygen uptake of individual pupae during the prepupal and pupal stages. Several readings were taken at specific intervals each day during the 4-5 days of pupation. No attempt was made to determine the specific respiration rates. From data obtained

with 20 nonlethal controls an "average" oxygen consumption curve was plotted as a standard for comparison. It resembles those described by Chen (Z. i. A.V. 84:38, 1951) and others, in showing a gradual decrease in oxygen consumption during the first half of pupation and an increase for the rest of the period. The respiration curves of six lethal strains, which have their period of action primarily in the prepupal or pupal stages, were then compared with the normal curve. In none of the cases was the ozygen consumption curve of the pupal lethal like that of the normal, in general form or in total oxygen uptake. Lethal N1A, which has its period of action in the late pupal phase, showed a gradual decrease in respiration rate, as in the normal, for the first half of the pupation period, but the decrease continued for the rest of the period. The total oxygen consumption was 80% of normal. In N51, N32, Co3A, and Co7 the over-all shape of the curves was similar to that of N1A but the initial rates were somewhat lower than normal and the decrease in oxygen consumption was greater. The total oxygen uptake was less than half that of normal. There appears to be a general correlation between the extent of development and the rate and amount of oxygen consumed. Although N61 is a "prepupal" lethal like N51, the respiration curve is quite different. During the first 1 1/2 days the oxygen consumption is equal to the normal but then there is no appreciable uptake the rest of the time and respiration is at a near-zero level. Since the number of pupae tested has b een small and the experimentation is in its initial stages, no conclusions will be attempted at this time.

Shima, T. Variation in triangular-black-spot patterns of the tergite in D. nigromaculata.

The second to fifth abdominal segments of D. nigromaculata are generally characterized by the presence of triangular black spots. Several variations occurred in black-spot patterns of the tergite among D. nigromaculata flies collected in the University Botanical Garden, Sapporo, during the period from May 1957 to September 1958. It was found after close study that the black-spot patterns are divided into seven types; A, B, C, D, E, F, and G. The results of a study of 739 specimens are summarized in the table.

No. flies observed	Types of pattern							Total
	A	B	C	D	E	F	G	
Male	13	77	136	80	10	37	33	386
Female	5	62	103	101	38	28	16	353
Total	18	139	239	181	48	65	49	739

It was found that type C was most frequent. There was no seasonal variation in pattern. The development of the black spots was also studied in flies after emergence. It was observed that the black spots stabilized their patterns 70 hours after emergence at 20° C. The question remains whether the variation in black-spot patterns is attributable to genetical or to environmental factors.

Sobels, F. H., D. Bootsma, and A. D. Tates. The induction of crossing over and lethal mutations by formaldehyde food in relation to stage specificity.

The induction of crossing over by formaldehyde food in male Drosophila larvae has been studied in correlation with stage sensitivity for the production of lethal mutations. The occurrence of bunches of identical or complementary crossover products was used as an indication that spermatogonial stages were sampled. In this way we attempted a further verification of Auerbach's conclusion that postspermatogonial auxocytes are most sensitive to the mutagenic action of formaldehyde given in the medium.

Immediately after hatching, first-instar larvae of the genetic composition +/dp b cn bw were placed on a medium containing 3% agar, 10% sugar, and 10% dry yeast, to which various concentrations of formaldehyde (BDH) had been added. Male flies hatching from such cultures were crossed with y sc^{S1} In-49 sc^{8}; dp b cn bw females. This stock permits one to test for crossing over in the second chromosome, as well as for lethals in the X chromosome; it was kindly placed at our disposal by Miss K. Nordback. To study the pattern of sensitivity in the treated testis, the males were given three virgin females per male at three-day intervals.

Of the different concentrations tested, we found 0.25% formaldehyde most effective in the induction of both lethal mutations and crossing over. Pooled data for comparable concentrations were 10% lethals in 1264 tested chromosomes in brood A, 3.5% lethals in 1532 chromosomes in brood B, and 0.9% lethals in 1506 chromosomes in brood C. This pattern of sensitivity, with highest frequency of mutation in the first brood, decline in the second, and a sharp drop in the third, is in all respects similar to that described by Auerbach and Moser (1953).

The table presents pooled data of five experiments on crossing-over induction.

	Brood A	Brood B	Brood C
Number of gametes	7449	17,832	25,895
Single recombinants	19	11	13
Crossover bunches	2	8	1
Crossing-over events	21	19	14
Percentage	0.28	0.106	0.054

The data show that crossing-over events (bunches of complementary or identical crossovers taken as one recombinational event) are significantly more frequent in brood A than in brood B (normal deviate $\chi = 3.026$; $P < 0.003$). The same is true for single recombinants ($\chi = 3.86$; $P < 0.001$). Stage specificity thus apparently also applies to the induction of crossing over by formaldehyde feeding.

In comparison to single recombinants, the number of bunches in brood B is significantly greater than in brood A ($\chi^2 = 5.64$; $P < 0.02$). Taking bunches as an indication of spermatogonial crossing over, these results agree well with Auerbach's idea that peak sensitivity to the mutagenic action of formaldehyde food is characteristic for a postspermatogonial stage of sperm development.

It is admitted that recombinations involving one marker only cannot be

distinguished from induced mutations at the loci under study. The distribution of the induced recombinations suggests, however, that in the present study we have been dealing mainly with induced crossing over; 10 were located between dp and b, 29 between b and cn, and 15 between cn and bw. The preference for the occurrence of chemically induced crossing over in the central part of the chromosome corresponds with findings of Whittinghill (1954) on crossing over induced by radiation.

Continued breeding tests showed that the great majority of all recombinant chromosomes is viable in homozygous condition. These data suggest a similarity between the processes involved in crossing over induced by chemicals in Drosophila males and those of normal crossing over in females. Since we did not free the second chromosomes from lethals before use in these experiments, the small remaining portion of recombinants that was homozygously lethal may have originated from pre-existing lethals.

Sokoloff, A. Studies of quantitative characters in D. pseudoobscura.

As part of a study of quantitative characters in populations of D. pseudoobscura derived from various localities in the distribution of this species, two problems have been investigated that may facilitate the work of other investigators using this species in similar problems. The first concerns the question whether the environmental conditions under which a given generation is reared will influence, by way of a maternal effect, any quantitative character measured in subsequent generations. The second is a practical problem: An investigator may lose a collection of adult flies from a particular locality between the time it is entrusted to the post office and the time it arrives at the investigator's laboratory several thousand miles away. Since the vials contain food, there may be eggs or larvae produced by one or many flies that have undergone a certain degree of starvation en route. If, on arrival at the laboratory, the material is transferred to fresh medium and the emerging flies are mass mated, will the quantitative characters measured in the group differ materially from those of flies reared under optimal conditions throughout their developmental history? In other words, will the data on quantitative characters obtained by this means be representative of the population of the particular locality?

To answer these questions, material collected at the Grand Canyon was transported by car to Cold Spring Harbor, New York. On arrival, female adults were introduced individually into culture bottles to insure the establishment of stocks. Half the bottles were placed in a 24° C incubator, the other half in a cool incubator maintained at 16° C. The medium from each of the two transporting vials (containing eggs and larvae of various ages) was transferred to two culture bottles and placed in the 24° incubator.

On emergence, the F_1 flies from these mass cultures were introduced en masse into oviposition bottles, young first-instar larvae were picked up from spoons containing medium, and introduced 30 per vial into 10 vials containing 4 cc of standard cornmeal-molasses-agar medium. All ten vials were placed in the 16° chamber. The F_2 adults emerging from the vials were aged for several days to allow complete hardening, then sacrificed; and the right wing and right hind leg were mounted in paraffin oil for later measurements.

From ten individual cultures maintained at each temperature, ten females from each of five cultures were mated to an equal number of males from the remaining five stocks in an oviposition bottle. Thirty young first-instar

larvae were introduced into each of twenty vials. Half of these vials containing F_2 larvae derived from 24° F_1 were placed in the 24° incubator and half in the 16° chamber. Similarly, half the larvae from 16° F_1 were reared at 24° and half at 16°.

The data for this experiment are summarized in table 1. A t value of 2.15 ($.02 < P < .05$) is obtained when the tibia length of F_2 females derived from 24° F_1 and reared at 24° is compared with that of F_2 females derived from 16° F_1 and reared at 24°. Since neither this character in males nor the other characters investigated in males and females derived from the two sources showed any significant difference, the anomalous value must be attributed to chance. It can be concluded that there is no maternal effect in the three characters investigated.

Turning to the second problem, the data are summarized in table 2. If we compare these data, for flies derived from F_1 that presumably were semi-starved to varying degrees during their early stages of development, with data for flies that received optimal food conditions but experienced a change in temperature (i.e., with the data for C F_2, table 1), we see that the results are nearly identical.

It should be noted that Prevosti (Cold Spring Harbor Symp. Quant. Biol. 20: 294, 1955), believing that the environment in which D. subobscura females had developed might influence quantitative characters of the F_1, decided to measure F_2 progeny. The results of the present experiments suggest that, in studies of quantitative characters of populations of D. pseudoobscura, it is not necessary to wait for the second generation to avoid a possible maternal effect associated with uncontrollable fluctuations of the environment in which the mothers are reared. These studies also show that, if adults are lost en route and if larvae are available in the mailing vials, the (F_1) adults emerging from them can be used to obtain quantitative measurements in the F_2 that will be characteristic of the population from which the sample was derived.

In D. robusta, Stalker and Carson (Evolution 3: 330, 1949), using Fisher's Discriminant Function (D_2), found a high D_2 value to be characteristic of a relatively northern phenotype. Samples of D. pseudoobscura have been obtained from different geographic localities and progeny reared under controlled conditions. Calculations of D_2 values are in progress.

(Supported by a grant from the National Science Foundation.)

Note: See following pages for tables 1 and 2.

Table 1

		N	$\bar{m}\pm$	S.E.	S.D.
			A F_2		
Males	WL	77	52.36	.07869	.6905
	WW	76	27.62	.04853	.4231
	TL	88	20.52	.03249	.3048
Females	WL	143	57.25	.06054	.7241
	WW	143	29.80	.03064	.3665
	TL	147	21.63	.01209	.1465
			B F_2		
Males	WL	113	52.52	.09934	1.056
	WW	113	27.63	.07160	.7611
	TL	113	20.82	.04237	.4504
Females	WL	102	57.97	.08485	.8570
	WW	102	29.87	.06912	.6981
	TL	102	22.11	.03757	.3795
			C F_2		
Males	WL	118	57.61	.07234	.7356
	WW	118	29.79	.06980	.7580
	TL	119	21.96	.03315	.3615
Females	WL	85	63.33	.03615	.3333
	WW	85	32.34	.07116	.6561
	TL	93	22.90	.05878	.5669
			D F_2		
Males	WL	130	57.38	.1882	2.146
	WW	130	29.66	.1019	1.162
	TL	130	21.88	.07819	.8914
Females	WL	107	63.75	.1163	1.203
	WW	107	32.23	.06489	.6710
	TL	107	23.10	.04628	.4785

A = F_2 reared at 24° from F_1 reared at 24°.
B = F_2 reared at 24° from F_1 reared at 16°.
C = F_2 reared at 16° from F_1 reared at 24°.
D = F_2 reared at 16° from F_1 reared at 16°.

WL = wing length
WW = wing width
TL = tibia length

Table 2

		N	$\bar{m}$	S.E.	S.D.
Males	LW	101	57.19	.08657	.9090
	WW	101	29.98	.05901	.6196
	LL	101	21.81	.03065	.3218
Females	LW	166	63.14	.08346	1.075
	WW	166	32.35	.04660	.6002
	LL	166	22.90	.03581	.4612

Sulerud, R. L. An investigation of possible aids for separating males of D. affinis subgroup species.

The tarsal segment index is proposed as the ratio of the lengths of the right first and second prothoracic tarsal segments, that is, first-segment length divided by second-segment length. This index was determined for males of five D. affinis subgroup species; seven of the strains were raised at the two temperatures indicated in the table. The mean ratio values and ranges for the different strains are shown in the table. In D. athabasca and D. azteca the first tarsal segment was always longer than the second (although several specimens of D. azteca were found in which the two segments were nearly equal). In D. tolteca and D. algonquin, on the other hand, the second tarsal segment was always longer; and this was also generally true for D. affinis (4 exceptions out of 100).

Further investigations may show that the tarsal segment index is useful for separation of males of certain pairs of the five species considered. Interspecific differences as to relative length of the first and second tarsal segments also exist in females of the D. affinis subgroup. In the strains studied, however, these differences were small (though sometimes significant), not affording separation; the females of all five species examined always had tarsal segment index values greater than 1.00.

The work of Hsu (1949) on the genital apparatus of male Drosophila indicated differences as to number of "primary teeth" on the "primary clasper" of the hypopygium among various species of the D. affinis subgroup (i.e., D. affinis, D. algonquin, D. azteca, and D. narragansett). In the present study the number of primary teeth was determined in males of laboratory strains of D. athabasca and D. azteca (60 males of D. athabasca and 60 of D. azteca, the strains having equal representation.) The genital clasper has right and left groups of teeth, and the ranges given below refer to the number of teeth in one group. However, since tooth number determinations were made separately for both groups, the ranges are based on the total variation within each clasper for the flies examined. The following are the tooth-number ranges observed. D. athabasca: Alaska (2 strains, College and Matanuska Valley), 8-11; Michigan (2 strains, Cheboygan and Iron Mountain), 8-10; New York, 8-10; Ontario, 8-9; Wyoming, 8-10. D. azteca: California, 4-6; Mexico, 4-6. In the few strains of each species examined, all D. athabasca males had 8 or more primary clasper teeth whereas no more than 6 such teeth were found in any D. azteca specimen. Although it was necessary to observe the claspers under rather high

magnification (ca. 100 X) in order to count the number of teeth, the relative sizes of the groups of teeth could be distinguished with a dissecting microscope (ca. 30 X).

Laboratory strains	18° C		27° C	
	n	Tarsal segment index values	n	Tarsal segment index values
D. tolteca Nicaragua	50	0.700±0.0050 (0.732-0.880)	50	0.823±0.0045 (0.754-0.875)
Bolivia	30	0.817±0.0179 (0,702-0.920)		
Mexico	30	0.792±0.0159 (0.716-0.870)		
Colombia	30	0.804±0.0076 (0.714-0.882)		
D. algonquin Wisconsin	50	0.823±0.0057 (0,735-0.886)	50	0.836±0.0070 (0.750-0.933
Nebraska	100	0.823±0.0087 (0.725-0.900)		
D. affinis Texas	50	0.875±0.0090 (0.763-1.002)	50	0.855±0.0094 (0.739-0.977)
New York	50	0.860±0.0052 (0.762-0.932)	50	0.930±0.0089 (0.830-1.08)
D. azteca California	50	1.13±0.0110 (1.03-1.22)	50	1.16±0.0035 (1.07-1.44)
Mexico	80	1.11±0.0049 (1.02-1.20)		
D. athabasca Quebec	50	1.24±0.0087 (1.12-1.39)	50	1.22±0.0083 (1.16-1.39)
Alaska	50	1.22±0.0057 (1.12-1.27)	40	1.23±0.0111 (1.12-1.31)

Suzuki, D. T. Investigation of the ruby locus for position pseudoalleles in D. melanogaster. Seven stocks of X-ray- and mustard gas-induced ruby alleles, kindly supplied by Dr. F. H. Sobels, were tested for pseudoallelism. Each mutant stock was crossed with a laboratory stock of ruby, and crosses were made between the mutant stocks in all possible combinations. None of the autosomes contained inversions. The alleles studied (Sobels, DIS-32) were rb^{16-4}, rb^{18-16}, rb^{18-8}, rb^{19-33}, rb^{26-23}, rb^{27-4}, and rb^{28-35}. Between 12,000 and 20,000 flies were examined in each cross and no wild-type progeny were recovered.

Taira, T., and S. Nawa. A note on pigmentation in the eye.

Previous reports have shown the important role of pteridine metabolism in eye-pigment formation in D. melanogaster. The localization of pteridine dehydrogenase in the abdomen was reasonably deduced from the absence of isoxanthopterin (IXP), derived from enzymatic oxidation of 2-amino-4-hydroxypteridine, in the head and its presence in the abdomen. Another hypothesis may be required to explain the reaction system of the enzyme in the eye.

Strain	Red eye pigment (OD_{500}/ml/mg)	Pteridine BFS in eye (μg/mg)	Pteridine IXP in testis (μg/mg)
Ore-R	22.1	16.5	24.1
BB	8.5	7.7	25.1
bar-3	7.6	6.8	23.8
L^2	5.1	3.8	23.2
Dp	5.0	4.2	26.5

An analysis was made with such mutants as BB, bar-3, L^2, and Dp. The relative amounts of red pigment are shown in terms of optical density at 500 micrograms per milliliter per milligram of whole-body wet weight, and relative amounts of pteridine are shown in micrograms per milligram of the same. The red pigment in those mutants obviously decreases in direct relation to reduction in eye size, and corresponds to a reduction in blue fluorescent substance (BFS) in the eye. The amounts of IXP in the testis, however, do not differ from those in normal strains. These results suggest an intimate relation between pteridine metabolism and differentiation of eye tissue; but the presence of similar metabolites of pteridine metabolism in the eye and in the testis, serving different biological functions, still remains a mystery.

Takada, H. Migration of some Drosophila in population tubes.

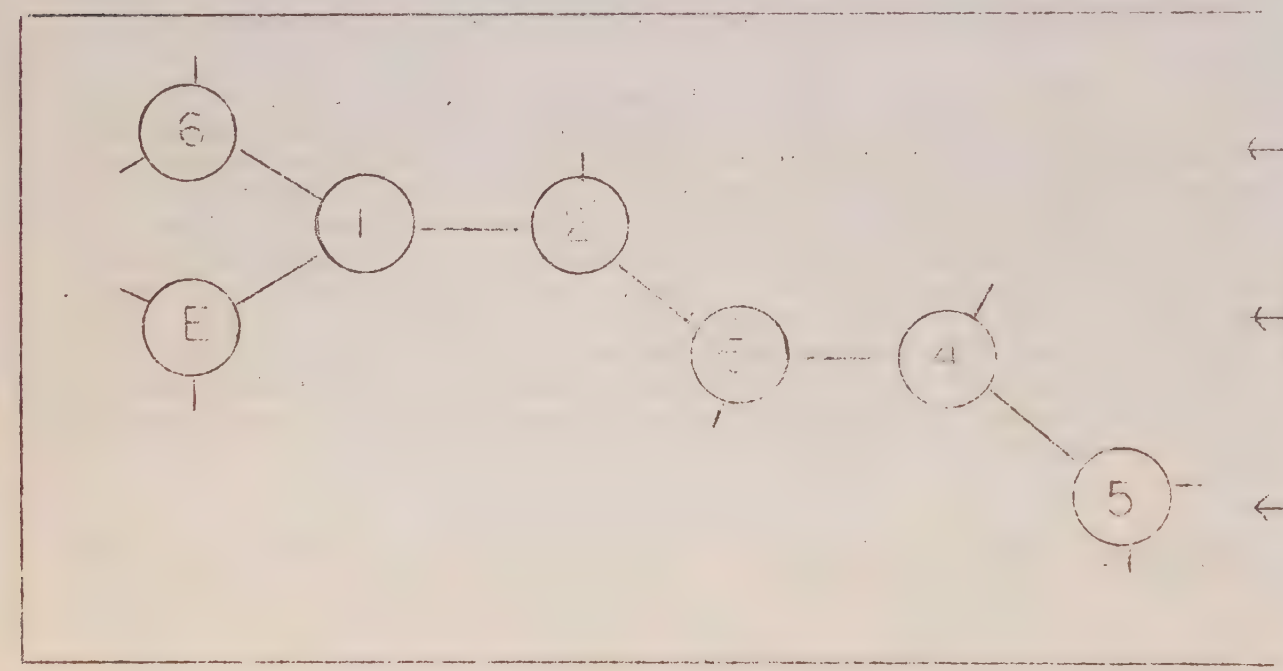
The population-tube idea was developed by Sakai (1956, DIS-30). According to him and his colleagues (1957), working with seven strains of D. melanogaster, the migration activity seems to be of a genetic nature. There are two kinds of migration in a population: one, called "random migration," takes place as the result of random movements of individual flies; and the other, called "mass migration," is caused by the pressure of population density. The present study deals with an analysis of migration in the following five species: virilis (Sapporo), funebris (Sapporo), nigromaculata (Sapporo), ezoana (Raus), and melanogaster (Taisetsu Mt.). The flies, collected from 1957 to 1958, were kept by random mating in vials in the laboratory for ten or more generations.

The experimental equipment consists of seven population tubes (illustrated in the accompanying diagram), each, except tube "E," containing about 2 cm of cornmeal-molasses-agar medium with yeast at the bottom. After flies had been kept for 12 hours in tube I, the six empty tubes were connected to it by vinyl-resin tubing. The test tubes were illuminated as shown in the accompanying sketch. Twenty-four hours later, the flies had

migrated to the other tubes. The experiment was repeated four times, at a temperature of 21° C.

There were striking differences among species with regard to level of population density: mass migration occurred in virilis at a critical density of 50-80, in funebris at about 250, in nigromaculata at about 30, in ezoana at about 50, and in melanogaster at 40-50. The intensity of random migration in funebris was 5.57% per day; in virilis it was 2.19%. It was observed that busckii was very active in random migration, whereas funebris usually showed weak activity in mass migration. The results seem to demonstrate that funebris has strong sensitivity in negative giotaxis and weak in negative aggretaxis.

As a rule, photokinetic migration in males and phototaxic plus photokinetic migration in females seem to b e responsible for the difference in behavior.



Takada, H., and Toyofuku, Y. Preliminary observations of the behavior of Drosophilidae during the winter.

Little has been known about the hibernation of drosophilid flies in Hokkaido. Field and laboratory observations were made by the writers in order to learn about the behavior of flies during the winter. Field observations in the University Botanical Garden, Sapporo, showed that the imagoes of D. auraria (A and B), D. nipponica, and Scaptomyza disticha survive the winter outdoors, forming colonies in the ground just beneath the snow. Observations were also made

of laboratory flies of seven species kept outdoors in special wooden boxes from December, 1957 to April, 1958. Pupae of D. auraria (A) and D. nigromaculata, and imagoes of D. funebris, survived the winter, whereas some other species died in the course of the experiment.

Takaya, H., S. Kaji, and I. Inouye. A new hereditary character of the compound eye in D. melanogaster.

A considerable number of facets in the middle of the anterior margin of the compound eye were found to be lost when larvae of wild-type flies of an Oregon stock were fed with soybean powder or monosodium glutamate (mixed with either molasses or Pearl's medium). Either of the two media brought about the eye deficiency. The feeding was tested for different lengths of time and during different periods of larval life. Administration of the substances during the period from 75 to 85 hours after hatching seems to b e effective in producing the deficient eye.

It was found that the eye deficiency brought about by the treatment is a hereditary character. Inbreeding of these flies always produced progeny with the same type of deficiency. Furthermore, in several successive generations of inbreeding the number of deficient flies and the degree of the deficiency showed a steady and gradual increase, although treatment with soybean powder or monosodium glutamate was not repeated in later generations. The number of flies with deficient eyes always amounted to 70%-90% in several generations of inbreeding. In later generations, no marked increase occurred, the percentage values being less than 95% throughout 36 generations examined. The eyes of the remaining 5% were quite normal, at least in external appearance. Their progeny, however, always presented the same type of eye deficiency, with frequencies of 5% to 50%, the values varying in the different generations from which tested flies were taken. Increase in the degree of the deficiency was gradual and was accompanied by an increase in the number of deficient flies. There appeared, in several generations of inbreeding, flies whose compound eyes were entirely lacking on both sides on account of complete failure of facet formation.

In crosses of these deficient flies with wild-type flies of Oregon or other stocks, eye deficiency was generally not produced in the next generation; but segregation always occurred in the F_2. If the flies used in the crosses had a very high degree of the deficiency, the character was reproduced even in the F_1 generation. We did not find any constant ratio of segregation of the character. The ratios actually obtained in F_1 and F_2 generations showed great variation in the different crosses examined. There was an apparent tendency, however, for flies with a higher degree of the deficiency to produce deficient progeny much more frequently than those with a lower degree.

A similar eye deficiency, in a very slight degree, has been found among wild-type populations of Oregon and several other stocks, although very rarely. These deficiencies of spontaneous origin showed the same mode of inheritance as the deficiency experimentally brought about through treatment with soybean powder or monosodium glutamate.

Tantawy, A. O., and M. Vetukhiv. Effects of size on fecundity, longevity, and viability in populations of D. pseudoobscura.

An experiment was designed to test the relation between wing length and lifetime egg production of adult females, egg viability, and longevity in populations of D. pseudoobscura kept at different temperatures. The flies were from two populations (cages 5 and 6) of D. pseudoobscura homozygous for the arrowhead gene arrangement in the third chromosome. These cages were maintained for two years at 25° C and 15° C, respectively. Samples of females selected for large, medium, and small wing length were kept at the same temperature as their initial cages, other samples at the reverse temperature.

The relation between female size and lifetime egg production is shown in table 1. Each sample consisted of 30 females. These results indicate

Table 1

Cage	Temperature (C)	Size	Egg production	Temperature (C)	Size	Egg production
3	25°	Large	33,948	15°	Large	60,642
		Medium	24,692		Medium	35,226
		Small	12,416		Small	21,666
6	25°	Large	33,837	15°	Large	66,841
		Medium	15,360		Medium	39,199
		Small	14,432		Small	26,706

clearly that the size of females has a definitely significant effect on their egg-laying capacity. Females kept at 15° C laid more eggs than those kept at 25° C. The analysis of coefficients of variation for wing length and egg production indicates that the latter character is affected more by environmental agencies than the former.

Table 2 shows the relationship between selection differential (S.D.) for wing length, average longevity, and average daily egg production per female. Standard errors for means are also presented. The unit of measurement is 1/100 mm. The results indicate that higher average egg production is associated with higher selection differential for female wing length and with longevity. It is of interest to note that the original temperature of the population may influence egg-laying capacity.

Phenotypic correlation coefficients between wing length and lifetime egg production indicate that these two characters are correlated to a highly significant degree. The correlations are: 0.5062 and 0.5401, and 0.2631 and 0.3228, for females from cages 3 and 6, respectively, at 25° and 15°. There are also highly significant correlations between thorax length, longevity, and egg production. Wing length has no effect on percentage of emergence and there is insignificant correlation between wing length and percentage of emergence. Table 3 shows numbers of eggs cultured, numbers of adults hatched, and sex ratio for groups selected for large and small size. Lifetime egg production is given for ten females in each selected group. The results indicate that percentage of emergence and sex-ratio depend on the genotypes of the parents rather than their phenotypes. The data of these experiments will be published soon.

Table 2

Cage No.	Temperature (C)	Size	S.D. ± S.E.	Average Daily Egg Production ± S.E.	Average Longevity ± S.E. (days)
3	25°	Large	21.68 ± 1.58	24.51 ± 1.41	37.31 ± 5.44
		Medium	2.83 ± 2.22	14.78 ± 1.27	35.20 ± 3.50
		Small	- 15.77 ± 3.68	12.57 ± 1.24	28.20 ± 5.13
6	25°	Large	25.19 ± 2.64	20.93 ± 2.08	36.30 ± 6.53
		Medium	4.78 ± 2.51	10.78 ± 1.22	35.18 ± 4.30
		Small	- 23.66 ± 3.44	10.53 ± 1.08	31.53 ± 9.88
3	15°	Large	21.67 ± 2.50	15.03 ± 0.76	119.12 ± 6.50
		Medium	6.04 ± 2.10	10.79 ± 0.57	95.00 ± 6.43
		Small	- 21.94 ± 2.06	7.33 ± 1.36	83.45 ± 9.91
6	15°	Large	26.14 ± 2.58	17.82 ± 0.81	92.91 ±11.45
		Medium	6.53 ± 4.42	15.18 ± 0.63	80.13 ± 6.26
		Small	- 19.23 ± 3.88	12.79 ± 0.64	70.00 ±10.69

Table 3

Cage No.	Temperature (C)	Size	Total Lifetime Egg Production	No. Hatched		% Emergence	Sex Ratio*
				Males	Females		
3	25°	Large	14,712	5,002	5,293	69.97	51.41
		Small	4,967	1,642	1,800	69.29	52.29
6	25°	Large	11,279	3,969	4,207	72.54	51.45
		Small	6,259	2,132	2,304	70.89	51.93
3	15°	Large	24,257	9,704	10,171	81.91	51.16
		Small	7,944	3,213	3,405	83.30	51.15
6	15°	Large	20,842	8,283	8,574	80.88	50.21
		Small	9,793	4,082	4,097	83.51	50.09

*Percentage of females.

Toyofuku, Y. Salivary-gland chromosomes of D. ezoana. Salivary-gland chromosomes of D. ezoana, a new species of the virilis species group from Hokkaido, were compared with those of D. virilis. The salivary chromosomes of D. ezoana are characterized by seven arms: two rod-shaped arms, a large V-shaped arm, a medium-sized J-shaped arm, and dotlike elements that apparently differ from those of D. virilis. There is evidence that the salivary chromosomes of D. ezoana are similar to those of D. littoralis (European form of the virilis group).

Tsukamoto, M. A new metabolic pathway of DDT in Drosophila.

It was reported in DIS-32 that D. melanogaster can metabolize DDT to a non-DDE derivative. The identification of this unknown metabolite has been successfully carried out. Results of paper and column chromatography show that the behavior of the metabolite is similar to that of a hydrol derivative of DDT, Kelthane /1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol/. The ultraviolet absorption curve of the metabolite also coincides with that of Kelthane. In alkaline condition the metabolite is converted into DBP (dichlorobenzophenone) and chloroform. The former can be detected by its absorption curve in ultraviolet regions, and the latter by the specific pink color with the Fujiwara reaction. From these evidences it has been concluded that in D. melanogaster DDT is metabolized not to DDE but to Kelthane. Such a metabolic pathway has not been known previously in either insects or mammals.

Ulrich, Hans, and D. Bassand. Relation between dosage and mutation frequency investigated by X-raying Drosophila zygotes.

The relation between dosage and mutation frequency was investigated by our new method of X-raying uncleaved eggs of D. melanogaster. Eggs from a cross of wild-type females with Muller-5 males were collected in large numbers by means of an apparatus similar to the one described in DIS-27, p. 124. These eggs were X-rayed with 200 to 1400 r (50 kv, 10 Ma; target distance varied from 96 to 43 cm so as to deliver the dose in 3 minutes) 10 to 20 minutes after being laid. One minute before irradiation the eggs were placed in a small chamber with an air current, where they were kept during irradiation. Embryonic and postembryonic mortality was recorded. The surviving F_1 females (+/M-5) were mated singly to their F_1 brothers (+/Y), which likewise had been irradiated as zygotes. Each F_2 offspring should consist of +/+ and M-5/+ females, +/Y and M-5/Y males. Absence or a frequency of less than 2.5% (as compared to the sisters of the corresponding phenotype) of either type of F_2 males indicates the induction of a recessive lethal in one of the X chromosomes of the irradiated zygote from which the F_1 mother in question developed. Hence in every F_2 offspring two different irradiated X chromosomes were tested.

The results of a large number of experiments are summarized in the table. They show that the mutation frequencies induced by doses of 200 to 1000 r agree fairly well with the assumption of a direct proportionality between mutation rate and dose.

The number of surviving F_1 adults was very small at doses higher than 1000 r: at 1200 and 1400 r only a few X chromosomes could be tested even after irradiation of many thousands of zygotes. Therefore the laborious experiment was repeated several times with the 1400-r dose level. In a first group of experiments the mutation frequencies were much lower than could be expected according to the direct-proportionality hypothesis. In a second group they did correspond to this expectation. It should be mentioned that in the second group the mutation frequencies in nonirradiated controls were higher than usual. There was a difference in experimental procedure between the first and the second groups of experiments. Since a very small number of larvae in the food is unfavorable to larval growth, the number of zygotes placed in one tube after X-raying was increased from 1000 in the first group to 3000 in the second one. If one assumes that females carrying a recessive lethal in one of their X chromosomes have a relatively higher mortality in

unfavorable than in favorable conditions, the resulting selection effect could account for the lower frequency of registered mutants in the first group of experiments. This question will be pursued further.

On the whole, there seems to be no reason so far to assume a real deviation of mutation frequencies from linearity. Dose levels higher than 1400 r could not be tested because beyond that mortality is nearly complete. The unusually high mutation frequencies recorded (e.g., 6.63% at 1000 r) demonstrate that the Drosophila zygote has very high sensitivity to the mutagenic action of X-rays.

(Work supported by Schweiz. Nationalfonds zur Förderung wissenschaftlicher Forschung.)

Dose	No. of experiments	No. of X-rayed eggs	No. of eggs per tube	Embryonic mortality (%)	Postembryonic mortality (%)	Total mortality (%)	No. of tested X chromosomes	Mutation rate (%)
200	2	6,900	400	35.4	21.5	49.2	2828	1.45
400	1	3,420	600	56.1	26.1	67.6	784	2.04
600	3	11,714	700	71.3	39.8	82.7	1646	4.50
800	1	8,140	800	82.4	42.3	89.8	682	4.99
1000	5	34,749	900	88.8	40.0	93.3	2052	6.63
1200	3	23,470	1000	90.6	68.7	97.1	546	4.76
1400	4	29,200	1000	92.3	78.2	98.3	386	4.92
1400	5	80,200	3000	95.0	63.7	98.2	1204	8.55
Controls (200 r to 1200 r):								
	12	4,200	200	12.0	10.0	20.7	1340	0.37
Controls (1400 r, first group):								
	4	2,194	200	13.4	14.2	34.5	492	0.20
Controls (1400 r, second group):								
	5	5,388	200	14.8	8.7	22.3	1802	0.61

Ulrich, Hans, and F. Würgler. Effect of anoxia on the frequency of mutations induced by X-rays in Drosophila zygotes.

In preliminary experiments (DIS-30, p. 155) we were able to confirm the well known fact that the biological effects of irradiation depend on the presence of oxygen and oxygen tension: the mutagenic action of X-rays on uncleaved D. melanogaster eggs was depressed by anoxia (produced by a nitrogen current) during irradiation. In pursuit of this problem F_1 eggs from a cross of wild-type females by Muller-5 males were X-rayed with 200 to 2000 r (50 kv, 10 Ma; target distance varied from 96 to 36.5 cm so as to deliver the dose within 3 minutes) 10 to 20 minutes after being laid. One minute before irradiation the eggs were placed in a small chamber with a current of pure nitrogen, where they were kept during irradiation. The experiments were performed simultaneously with the ones described in the foregoing note (Ulrich and Bassand); that is, every day the same dose was

administered in air and in nitrogen (2000 r in nitrogen only) in order to obtain comparable results. Embryonic and postembryonic mortality was recorded and the rate of sex-linked recessive lethals was determined by pair-mating surviving F_1 adults (+/M-5 x +/Y) and scoring for presence or absence of either type of males (+/Y or M-5/Y) in the F_2 progeny.

The table shows the summarized results of several experiments. Over the whole range of doses applied there was a direct proportionality between dosage and mutation rate. In nonirradiated controls the nitrogen-treated moiety had in general a somewhat higher mutation frequency than the portion exposed to an air current. At doses between 200 and 1000 r, nitrogen reduces the mutation frequency to about 50%. Whether this is true also for levels of 1200 and 1400 r is not yet clear because of the variation in mutation frequency after irradiation in air mentioned in the foregoing note.

(Work supported by Schweiz. Nationalfonds zur Förderung wissenschaftlicher Forschung.)

Dose	No. of experiments	No. of X-rayed eggs	No. of eggs per tube	Embryonic mortality (%)	Postembryonic mortality (%)	Total mortality (%)	No. of tested X-chromosomes	Mutation rate (%) in nitrogen	Mutation rate (%) in air*
200	2	5,780	400	20.6	16.7	33.9	2864	1.08	1.45
400	1	2,996	300	31.1	16.4	42.4	1270	1.89	2.04
600	2	8,540	400	49.6	29.9	64.2	2680	1.94	4.50
800	1	5,980	400	53.9	25.0	65.4	1622	2.59	4.99
1000	2	8,325	900	62.7	25.5	72.2	872	3.92	6.63
1200	2	8,760	1000	74.4	46.0	86.1	960	3.96	?*
1400	3	6,740	1000	80.1	48.3	89.7	492	4.47	8.55 ?*
2000	2	18,000	1000	88.6	72.8	96.9	512	9.24	-
Controls	14	6,496	200	11.4	10.0	20.2	1840	0.49	0.33

*See Ulrich, H., and D. Bassand, foregoing note.

Ursprung, H. Nonautonomy of the eye-color mutant bronzy (bz, 1-64.9, Fahmy, DIS-32).

In this issue of DIS Glassman reports that the mutant bz is an allele of maroon-like (ma-l). He bases this conclusion on genetical and biochemical evidence. Transplantation experiments (E. Hadorn, unpublished) have revealed nonautonomy in the mutant ma-l insofar as implantation of wild-type tissue into ma-l larvae normalizes the drosopterin content of the hosts and causes the formation of isoxanthopterin, which normally is absent in ma-l.

In order to have another criterion for the possible allelism of ma-l and bz, experiments were carried out to check for nonautonomy in bz. For this investigation we used a vbz stock (orange eyes) kindly put at our disposal by Dr. Glassman. When wild-type Malpighian tubules were injected into vbz larvae, the eyes of the adult hosts definitely were altered towards red,

owing to an increase in drosopterins by comparison with uninjected controls, as determined by the paper chromatographic method of Hadorn and Mitchell (Proc. Natl. Acad. Sci. U.S. 37: 650-665). At the same time the host testes contained isoxanthopterin, which is normally absent in vbz. The control experiment (vbz Malpighian tubules transplanted to vbz hosts) failed to reveal any influence of the implant on the pteridine pattern of the host. In another experimental series we implanted bz eye Anlagen into wild-type hosts. The amount of drosopterins in these implants was definitely higher than in control implants (bz Anlagen transplanted into bz hosts) or in uninjected bz controls. Thus bz, like ma-l, is nonautonomous with regard to formation of drosopterins and isoxanthopterin.

Zimmering, S. Presence of the normal allele of su-f in the B^S segment of $sc^8.Y.B^S$. F_1 male offspring, from crosses of y f:=/$sc^8.Y.B^S$ females by y v f car su-f males carrying a normal Y chromosome, were phenotypically y^+ v f B^S car. That is, $sc^8.Y.B^S$ (Brousseau and Lindsley; Brousseau; DIS-32), in addition to carrying y^+ and B^S, also possesses the normal allele of su-f. Tests to determine whether su-f^+ was associated with the sc^8 tip in a chromosome of type sc^8 showed that it was absent. It may be inferred, therefore, that the B^S segment of $sc^8.Y.B^S$ includes su-f^+.

This finding has provided a simple explanation of certain puzzling results from the following experiment. From the mating of gamma-irradiated sc^8 w/$sc^8.Y.B^S$ males with y w^{m4} females, phenotypically y B^S females and y^+ w^{m4} B^{S+} males were recovered as exceptions from treated spermatogonial cells. These classes of offspring were presumed to arise from an exchange between some portion of Y^L, which carries B^S, and the heterochromatic region near the left end of the sc^8 X chromosome, with the subsequent recovery of the B^S y^- X chromosome in an F_1 female, and of the Y chromosome, now carrying a sc^8 tip on Y^L as well as the one already present on Y^S, in an F_1 male. However, exchanges between Y^L (unlike Y^S) and the distally placed heterochromatin of an X chromosome of this type do not ordinarily occur. It would be expected that if they did occur, the consequences of such exchange would be that the free Y chromosome recovered from the exchange would nearly always lack fertility factors of Y^L, and that the deficient X chromosome would lack fertility factors of Y^S and nearly always have those of Y^L. In that case, the y^+ w^{m4} B^{S+} males, which would lack Y^L fertility factors, should be sterile. Surprisingly, of 71 such males recovered, 67 proved to be fertile. Moreover, as expected, males carrying the deficient X chromosome, when supplied with $sc^{Vl}.Y^S$, which contains material covering the deficiency, are proving to be sterile. The exchange, therefore, probably occurred proximally to the B^S marker but distally to the fertility factors of Y^L, so that all the necessary fertility factors normally present on Y^L remained associated with this arm despite its apparent involvement in an exchange with X heterochromatin. In the light of the evidence indicating the presence of su-f^+, known to be located in the most distal portion of the proximal heterochromatic region of the X, the simplest interpretation of all these results is that the exchanges were between the large, distally placed heterochromatic portion of the sc^8 X chromosome and a portion of X heterochromatin intercalated between the marker B^S and the most distal fertility factors of Y^L. The finding by Dr. Muller that the Y chromosome carried by the fertile y^+ w^{m4} B^{S+} males results in the kind of hairy-wing effect, observed when two sc^8.Y chromosomes are present in the male, supports the inference that

this newly derived Y chromosome is probably of the constitution $sc^8\ Y^S.\ Y^L\ sc^8$. These Y chromosomes will be tested to determine what proportion of the exchanges occurred to the left and to the right of $su\text{-}f^+$.

The method employed by Brousseau and Lindsley to obtain the preliminary $Y.B^S$ chromosome that was later used by Brousseau to make the $sc^8.Y.B^S$ by simple exchange with a $sc^8.Y.bw^+$ involved, as a first step, the recovery of a $B^S\ car^+\ Y^L.Y^S$ crossover product from an exchange between the proximal fragment of chromosome B^S, marked with B^S car, and Parker's $XY^L.Y^S$. This origin indicates that the region containing $su\text{-}f^+$ was already present in the $XY^L.Y^S$ chromosome, distal to Y^L, was present again in the derived $B^S\ car^+\ Y^L.Y^S$ chromosome, and remained present after the deletion of the latter that gave rise to the $sc^8.Y.B^S$ chromosome.

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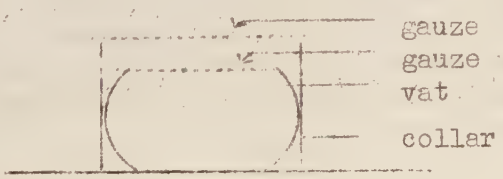
TECHNICAL NOTES

Abrahamson, S. The Heinicke commercial glassware washer for Drosophila bottles and vials. We have recently installed the electrically heated Heinicke typhoon washer in our laboratory. This small model can wash and rinse 121 vials every 10-11 minutes at 175° F, and 25 bottles every 15-20 minutes at 190° F, including loading, unloading, and reheating time. In order that the machine may perform efficiently, however, the cultures are flushed with water after being autoclaved, and the contents (mainly food and cellucotton) are dumped. The machine washes the glassware cleaner than can be done by hand, and saves time because the operator can perform other chores during the washing cycle.

However, there is a bug in the machine's operations in addition to the Drosophila. When the bottles are heavily covered with pupa cases, the cases lodge in the openings of the jets when the wash water is recycled and prevent full washing efficiency. To obtain full washing efficiency the jets have to be cleaned after each cycle, which is time consuming and a nuisance. Dr. Kurt J. Heinicke, president of the company writes: "...we will experiment with a screen to be placed on top of the present debris tray to catch the pupa cases. This screen could easily be lifted out after each cycle and emptied. It would be easily accessible and its emptying would be a matter of seconds." If this modification proves successful I would have no hesitation in recommending this washing unit.

Altenburg, E., and L. S. Browning. The vat method of culturing Drosophila. Mass cultures of flies grown in glass vats (8 inches in diameter and 3 inches high, with a layer of well-yeasted food about 3/4 inch in depth) yield large numbers of offspring (about 2000) when about 200 parents (100 males and 100 females) of normal fertility and vigor are added to the vat. The vat is covered with a double layer of gauze held in place by a stout rubber band. A metal collar, 5 inches high, covered with gauze, is made to fit snugly over the vat (see figure). The collar is slipped all the way to the base of the vat and serves as an over-all cover (its top being about two inches above the lower covering of gauze). This second covering prevents the escape of any flies that may have crawled as larvae through the first covering, and also fully protects the vat against contamination. As a rule, not many larvae crawl through the lower covering of gauze, and they do so only if there is overcrowding. Their number can be reduced by reducing the number of parents. In any event, none get through the second covering.

Fresh yeast suspension is added to the food on the third day. This is done after the parents have been etherized (by inverting the vat over a piece of towel paper saturated with ether) and the flies removed from the gauze onto which they fall. The parents can then be used to start a fresh brood. On the seventh day the vat is again examined and water is added if the food seems too dry. As many as ten broods at 3-day intervals can be made, the total yield from a vigorous stock being from 20,000 to 25,000 flies.



Bennett, Jack. An inexpensive, simple etherizer for classroom use.

A simplification of an etherizer reported by Lloyd L. Arnold (Amer. Biology Teacher 19: 248-251) has proved very useful in the student and research laboratory. It consists of a polyethylene bottle of the type used to dispense catsup, mustard, or salad dressing, which has a long (3 cm) pointed spout with a small (approx. 1 mm) hole in the end. The bottle is loosely packed with cotton, 5 cc of diethyl ether is added (sufficient for several hours' use), and the cap and spout are replaced. In use, the vial or bottle containing flies is gently inverted and the spout is carefully inserted past the cotton plug; ether vapor is expelled into the vial or bottle by pressing the sides of the etherizer. CAUTION: Remove spout before releasing sides of the etherizer or the flies may be drawn into the etherizer. When the flies succumb they fall onto the cotton plug, which can then be removed and the flies shaken off for examination. The polyethylene bottles cost about 25 cents (and are often thrown away by restaurants and housewives), and are virtually unbreakable. They require much less ether than most other types, and are adaptable to almost any type of container, including the polyethylene population cages.

Braver, Gerald. Orcein.

Gurr's Orcein, recommended by B. M. and H. Slizynski in their Technical Note in DIS-32 (page 171), may also be obtained from: ESBE Laboratory Supplies, 459 Bloor Street West, Toronto 4, Ontario, Canada.

Burdick, A. B. Various uses for da.

A. E. Bell's gene da, daughterless, in D. melanogaster (DIS-28: 73) has two interesting uses that may be of value. By substituting da in different stocks containing pseudoalleles in the sex chromosome one can obtain females homozygous for daughterless, da/da, and transheterozygous for the pseudoalleles in question. These females will yield only male progeny, which then can be screened for recombination between the pseudoalleles. In addition da is of value in mutation studies. A mating of XX females, homozygous daughterless, da/da, to irradiated males will yield only male progeny, carrying the irradiated sex chromosome. This type of mating eliminates the necessity of sorting out the males from a large offspring consisting of both sexes, and it also results in a larger yield of males in a given bottle.

Farnsworth, M. W. Procedures for the isolation of mitochondria from adult and larval Drosophila.

Our work has entailed the spectrophotometric assay of mutants for enzyme activity, and it has been found necessary to utilize isolated mitochondria for this purpose. The isolation procedure may be helpful to others faced with similar problems. We have successfully isolated mitochondria both from larvae (second and third instar) and from adult thoraces by procedures modified from that used by Sacktor with houseflies (J. Gen. Physiol. 37: 343, 1954).

Procedure for adults: Adults are isolated daily, allowed to feed in fresh food vials, and used three days after eclosion. Carbon dioxide anesthesia is as follows. Chopped dry ice is placed in a stoppered sidearm flask. Rubber tubing ending in a glass tube is attached to the sidearm.

The flies are shaken into empty dry vials, around 30 per vial, and the tube from the sidearm flask is inserted into the vial. The flies are almost immediately anesthetized, but should remain in contact with the CO_2 for several minutes. They are then shaken onto a plate beneath the microscope and with forceps the heads and abdomens are removed. Even with lengthy exposure to CO_2, only about 20 flies can be handled at a time before recovery from anesthesia. The thoraces are placed in approximately 1.5 ml of ice-cold 0.25 M sucrose, contained in a small mortar which is kept in a bowl of cracked ice. Usually 150 flies of mixed sexes are used for an experiment. After the thoraces have been collected, they are thoroughly ground in the mortar. A glass homogenizer can also be used. After grinding, the brei is filtered under suction (a water pump is sufficient) through a pad previously moistened with the sucrose solution. The pad is composed of cotton covered on either side with several layers of cheesecloth, the whole being placed in a small Buchner funnel. The filtrate is collected in a small sidearm flask placed in a bowl of ice. The mortar is rinsed twice with a total volume of around 10 ml sucrose, and the pad is washed with another 20 ml sucrose solution. The filtrate is poured into a 40-ml centrifuge tube and the flask rinsed with another 5 to 10 ml of the sucrose solution. The filtrate is centrifuged twice in the cold at around 1000 X g to remove yeast, nuclei, broken cells, and debris. After this step, the material is centrifuged at around 16,000 X g for 15 minutes in the cold to precipitate the mitochondria. The sucrose supernatant is then poured off and replaced with 15 ml ice cold 0.9% KCl. The mitochondrial pellet is resuspended and recentrifuged. Three such KCl washes are carried out. The final precipitate is resuspended in an appropriate volume of 0.9% KCl (e.g., around 1.0 to 1.5 ml) and aliquots are removed for spectrophotometric assay and nitrogen or protein determinations. If relatively small cuvettes are used, the aliquots need not be greater than 0.1 ml each, and thus a number of runs can be made on the same sample.

Procedure for larvae: In most respects, the procedure with larvae is identical to that given above. If times stages are desired, eggs are collected, and 24 hours after hatching the larvae are transferred to standard food in Petri dishes, approximately 120 per dish. At the age desired, larvae are removed from the dishes, washed twice with distilled water and transferred to an ice-cold mortar containing around 1.5 ml sucrose solution. The subsequent procedure is the same as that outlined above. As a general rule, we use 150 third-instar larvae (72 hours after hatching) per experiment. For second instar (48 hours after hatching), around 500 larvae are required.

Forbes, Clifford. Improved method of preparing Drosophila food.

A 5-quart thermostatically controlled electric saucepan makes an excellent unit for preparing Drosophila culture medium. The unit used in the writer's laboratory has a detachable thermostatic element, so that the pan is easily cleaned. The automatic control permits rapid cooking of the medium without boiling over or scorching. The cost of the pan with control is comparable to that of a good hot plate of similar wattage and an aluminum pan.

Gottschewski, G. H. M. A synthetic medium for tissue culture of imaginal eye discs of the third instar.

Eyes that are normal by comparison with eye Anlagen growing in situ can develop in vitro from explanted Anlagen of D. melanogaster. Imaginal discs, including

ring gland and bulbus opticus, were explanted from third-instar larvae. Shape and size of the cornea, the diptic and neural apparatuses, the protocerebrum--particularly the lobus opticus with medulla externa and interna--and the chiasmata are normal.

The synthetic medium was composed of the following substances.

Amino acids:

L-arginine	17.0 mg	L-histidine	19.6 mg	L-phenylalanine	20.0 mg
L-cysteine	4.0 mg	L-leucine	20.0 mg	L-threonine	12.0 mg
L-cystine	0.3 mg	L-lysine	14.0 mg	L-tryptophan	2.0 mg
L-glycine	1.2 g	D-methionine	5.0 mg		

Carbohydrates:

D-glucose	0.16 g
D-mannose	0.16 g
D-fructose	0.16 g

Vitamins and Coenzymes:

choline	0.5 mg	pantothenic acid	0.1 mg	DPN	1.0 mg
folic acid	0.05 mg	aneurine	0.05 mg	TPN	0.5 mg
inositol	0.1 mg	lactoflavin	0.05 mg		
niacinamide	0.5 mg	pyridoxine	0.1 mg		

Purines and pyrimidines:

adenine deoxyriboside	0.05 mg	thymidine diphosphate	0.05 mg
guanine deoxyriboside	0.05 mg	adenosine triphosphate	0.5 mg
uridine-5-triphosphate	0.05 mg	RNA	5.0 mg
deoxycytidine diphosphate	0.05 mg	DNA	5.0 mg
deoxy-5-methyl cytidilic acid	0.05 mg		

Peptides and proteins:

glutathione	0.05 g	glycyl glycine	0.1 g
peptone	0.1 g	casein hydrolysate	0.5 g

Minerals:

NaCl	0.5 g	$MgCl_2.6H_2O$	0.03 g	$NaCo_2CH_2$ (sodium acetate)	5.0 mg
$Na_2HPO_4.5H_2O$	1.0 g	$MgSO_4.7H_2O$	0.03 g	$CaCl_2.2H_2O$	2.0 mg
KCl	0.03 g				

Yeast extract: 1.5 mg

H_2O (double distilled): 100 ml

pH: 7.0-7.2

Hanks, George D. A simple electrical device for counting two classes of Drosophila.

A counter consisting of a telegraph "bug" and two electrically operated counters has been used for several months in classifying sex. Flies may be classified accurately without being separated. A telegraph bug is an instrument made of a lever and two contacts: when the lever is pushed one way, one contact is made (thus recording one count on the first counter), and when the lever is pushed the other way the other contact is made

(recording a count on the second counter. The counters may be obtained from Herbach and Radoman, Inc., 1204 Arch Street, Philadelphia 7, Pa. for $15.50 each. This system is highly recommended for use when there are only two distinct classes to be counted. With a little practice one can make counts with speed and accuracy hardly matched by any other device.

Newstead, Ninita. Xylol-hardening technique for developmental study of whole-wing mounts.

Flies were bred in culture bottles heavily inoculated with yeast. Pupae collected while the puparium was still white were incubated in Petri dishes at 25° C on filter paper moistened with a thin suspension of yeast. At the desired time, pupae were fixed in 100% alcohol for 10 minutes, hardened in xylol for 1/2 hour, rehydrated through descending concentrations of alcohol, and dissected in water. The wing was then stained in aceto-orcein for 15 minutes, dehydrated through ascending concentrations of alcohol, cleared in xylol (3 changes, 5 minutes each), and mounted in Canada balsam or Euparal. It was found that hardening the pupae in xylol prevented disintegration when the puparium was removed.

Nicoletti, Benedetto. An efficient method for salivary-gland-chromosome preparations.

We think it worth while to make available to other workers the method of salivary-gland-chromosome squash preparation used by J. Schultz, since it is not currently to be found in the literature. We have routinely employed this method, with slight modifications, and have found that it has many advantages, particularly simplicity and reproducibility.

The procedure is as follows:

1) Dissect larvae (grown at 18° C on food enriched early in larval life with extra yeast) in a drop of Shen's solution[1] on a siliconed slide.[2]

2) Transfer the glands immediately into a drop of 45% acetic acid placed on the same slide. Allow to remain for 1 minute.

3) Transfer the glands to a small drop (2-3 mm in diameter) of lactic-acetic orcein[3] stain on the center of the slide, and leave for 10 to 15 minutes.

4) Place a siliconed coverslip (22 x 22 mm) over the preparation. Hold down with the tips of two fingers of the left hand. With a small rubber hammer tap the cover glass several times directly over the glands (we use the ball end of a toy ball-peen hammer obtainable from a dime store).

5) Fold a sheet of bibulous paper closely around the slide and press well with the thumb in a rolling motion, being careful not to let the cover glass slip.

6) The coloration is complete after 24 hours, but the slides can be observed immediately to check the spreading of the chromosomes. If the spreading is not sufficient, pressure may be reapplied. Microscopic examination and photography are improved by phase contrast and green filter.

The preparation can be maintained for several weeks without sealing owing to the low water content of the stain. If permanent slides are

desired, staining should be performed on a siliconed coverslip; the smearing is then accomplished by inverting the coverglass on an unsiliconed, well-cleaned slide. Use of an unsiliconed slide assures that the preparation will adhere to the slide when the coverslip is subsequently removed. The final step is to make them permanent by the dry-ice technique.[4]

The main advantages of this method are several. The use of a stain of low volatility prevents drying during the stain procedure and makes sealing unnecessary. The siliconed slides, being hydrorepellent, retain the solution as discrete droplets, facilitating dissection and staining. Furthermore, the silicone film greatly improves the spreading of the chromosomes.

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[1] 9 g NaCl, .42 g KCl, .25 g $CaCl_2$, 1 liter H_2O.

[2] To make siliconed slides dip the clear slides in undiluted G.E. SC-87 Dry-Film Silicones for a few minutes, working under a hood. Then wash the slides with hot water and soap, rubbing each one to remove the greasy film. Dry with a clean cloth. The slides should appear as normally clean slides, though they will retain a molecular film of silicone that makes them hydrorepellent. Siliconed coverslips can be made in the same way: to dip them in the silicon we use the holders described by R. C. von Borstel and D. L. Lindsley, Stain Technology 39: 23-26, 1959. Siliconed slides and coverslips can be reused as long as they maintain their hydrorepellent properties.

[3] 2% by weight of powdered orcein in equal parts of 85% lactic acid and glacial acetic acid. (Orcein Natural, C. T. Gurr Ltd., London, England.) (Editor's note: Also obtainable in Canada; see Technical Note by Gerald Braver.)

[4] W. K. Baker, DIS-26: 129, 1959.

Rapaport, Sarah. An improved etherizing cup.

The repeated etherizing of flies, while handling large scores, is simplified by slight improvements on the standard etherizing cup. Eight to ten holes are bored in the upper third of the plastic cup (bottom up) with the aid of a hot wire (diameter ca. 1 mm). The holes allow slow air circulation into the cup, and the flies are kept alive and well etherized for a period of over fifteen minutes. If some stirring is observed through the transparent material of the cup, however, it is possible to administer more ether without moving the cup. This is done through a large hole (diameter ca. 8 mm) in the center of the bottom of the cup, immediately above the cotton wool, gauze, or whatever absorbent is used. In this fashion flies can be kept alive and etherized for well over half an hour. The possibility of physiological or reproductive damage arising from prolonged etherization has not been investigated. When flies begin to stir during the scoring it is also very handy to cover them with the cup, before applying ether through the upper hole.

Stevenson, Richard. A new bait-container for collecting wild Drosophila.

Although the common 50-pound lard can is still considered one of the best containers for bait to attract wild species of Drosophila, we have encountered some difficulty in preventing loss of the cans to pilferers in

easily accessible localities. We have adopted the 5-gallon fiber container in which ice cream is supplied to dealers. These containers are fitted with lids, are very light in weight, can be nested, and are relatively inexpensive, costing about 30 cents each. The average life of a container is about two months under field conditions but can be increased somewhat by applying shellac or paraffin to both surfaces. Any ice cream manufacturer can supply the containers in quantity.

Strickberger, Monroe W. An improved salivary technique. A useful technique for preparing and staining salivary glands has been modified from one used in Mainx's laboratory (communicated through O. Pavlovsky at Columbia University). The addition of lactic acid to ordinary aceto-orcein improves chromosome spreading greatly, so that diagnosis of arrangements and inversions can be made with increased facility. In addition, slides can now be kept unsealed, at room temperature, for more than a week, and even longer when sealed with paraffin. So far, sealed slides kept at 19° C have remained in good condition for over two months. The concentration of lactic acid now being used for D. pseudoobscura preparations is 15%. The glands are dissected out in the aceto-orcein-lactic acid solution, placed in a drop of the same solution on a clean slide, then separated and squashed in the usual way. At the 15% concentration of lactic acid we are able to get nine glands under a regular coverslip (22 mm square) without any overlapping. When the concentration of lactic acid is increased (to 25%, 50%, and 75%), the degree of chromosome spreading is correspondingly increased, a procedure useful for species with recalcitrant salivaries such as the willistoni group. We make 100 cc of the 15% lactic acid solution by dissolving 1 gram of orcein (Gurr) into 51 cc of hot glacial acetic acid, adding 34 cc of distilled water and 15 cc of lactic acid (Merck 85%), and then filtering. The keeping quality of the solution is excellent.